Title: NC 00573 Management Strategies for Tobacco Insect Pests

Project Leader: D. Michael Jackson

I. Summary of Research:

A. Host Plant Resistance Studies.

1. Field Experiments.

(a.) Three replications of 154 Nicotiana tabacum entries (varieties, breeding lines, and tobacco introductions) were planted in 12-plant plots at Tifton, Ga. and Oxford, NC in a randomized complete block design. Natural pest and beneficial insect numbers and damage were monitored several times over the growing season. Leaf punches (ca 2-cm diam), taken from 6-week-old plants, were dipped into methylene chloride in scintillation vials. These samples were frozen immediately on dry ice and shipped to Athens, GA for analyses of cuticular chemical components by cooperating USDA, ARS Research Chemists. Data from this screening program are used to identify new sources of insect resistance, and to quantify relationships between insect resistance and levels of cuticular components on green leaves.

(b.) Natural populations of beneficial and pest insects were monitored in field plots containing 70 entries representing 65 Nicotiana species. Entries were planted in 3 replications of 12-plant plots in a randomized complete block design at Oxford and at Tifton. Green leaf samples were taken from each entry for analyses of leaf surface chemicals, as previously described. Insect resistance and cuticular chemistry among the Nicotiana species varied widely.

(c.) Two susceptible (NC 2326 and Speight G-33) and two tobacco hornworm-resistant (I35 and TI 1112) tobacco entries were planted in three replications of 12-plant plots at each of three locations: Oxford, Tifton, and Florence, SC. Plants were examined every 2 days for 2-3 weeks, and all hornworm eggs were counted. At Oxford, the location of each egg on the plants was recorded. Leaves were sampled for leaf surface chemicals, as previously described, on the first and on the last day of this experiment. The insect-resistant entries (11%) received significantly fewer naturally deposited hornworm eggs than on the two susceptible entries (89%). Nearly 86% of the eggs were laid on the lower leaf surface, and ca. 75% of the eggs were oviposited on the upper five leaves of the plants. The two resistant entries have very low levels of cuticular diterpenes and fatty alcohols found on the flue-cured varieties.

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(d.) Five tobacco entries (NC 2326, TI 165, TI 1024, TI 1223, TI 1112) were planted at Oxford so that the cuticular chemistries of their flowering parts could be investigated. The leaves of these entries vary greatly in their cuticular constituents, and they encompass the range of components found on Nicotiana tabacum. Flowers were picked and brought into the laboratory where they were sampled by dipping into scintillation vials containing methylene chloride. Some of the flowers were dissected and the individual flower parts were sampled. The following treatments for each of three replications were dipped in methylene chloride: (1) 50 intact flowers before opening, (2) 50 intact flowers just after opening, (3) 50 flower stems, (4) 25 leaves from the flower stems, (5) 50 sepals, (6) 50 closed corollas, (7) 50 open corollas, (8) 50 anthers, (9) 50 styles + stigmas, and (10) 50 ovaries. In addition, three replications of fresh frozen samples were taken from the above treatments. The chemical data have not been fully analyzed at this time, but both cuticular components and internal constituents will be determined. These studies were conducted because it is known that Heliothis moths prefer to oviposit on the flowering portions of the plant, and we want to determine which chemical constituents contribute to their ovipositional behavior.

2. Oviposition Cage Experiments.

Eighteen outdoor screen cages (2.4 x 2.4 x 2.0 m) were used for several experiments on tobacco budworm and tobacco hornworm ovipositional behavior. These enclosures had plywood floors and gabled roofs that extended beyond the cage walls. Two 7.5 w light bulbs on a rheostat were used to maintain proper night-time lighting in each cage. For an experiment either 2 or 4 test plants in pots were placed on a board nailed diagonally across one corner of a cage 1.0 m above the floor. A similar number of check plants were placed on another board across the opposite corner of the cage. Either 10 tobacco budworm or 6 tobacco hornworm female moths were placed in each cage. The next morning all eggs were counted on the plants. Each cage-night served as one replication of a particular experiment.

(a.) All 70 entries of the Nicotiana species were seeded in a greenhouse, and then transplanted to 5-inch pots. They were moved outside under some large pine trees after about 2 weeks. Enough plants survived from 55 of these entries so that they could be tested for tobacco budworm and tobacco hornworm ovipositional preference. For each experiment, 4 test plants were paired against 4 NC 2326 control plants. Each plant was used in 2 replications for each insect type. These plants were saved for tobacco budworm larval feeding studies. We tried to get 10 replications for each entry for each insect. Leaf punches for chemical analyses were also taken from two replications of each Nicotiana species. Ovipositional response by tobacco

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budworm moths ranged from 0.1% of the eggs on N. glauca (i.e. 99.9% on NC 2326) to 62.3% on N. trigonophylla (37.7% on NC 2326). A similar range was observed from the tobacco hornworm experiments. Chemical data suggest that Nicotiana species containing high levels of duvane diterpenes or sugar esters are heavily oviposited on by tobacco budworm moths, while species with low levels of these components are resistant to tobacco budworm oviposition. Those with glabrous leaves are also resistant to tobacco hornworm oviposition.

(b.) Further studies were done on the activity of duvane diterpenes (α- and β-4,8,13-duvatrien-1-ols and α- and β-4,8,13-duvatriene-1,3-diols) on tobacco budworm oviposition. Duvanes were removed from the leaves of commercial flue-cured tobacco cultivars by dipping whole plants in beakers of methylene chloride. These chemicals were isolated and purified by chemists at Athens, GA. They were the bioassayed in the oviposition cages described above after being sprayed onto nonhost plants. During 1984, sweet corn and lima beans were used as the plant substrate. This year duvanes were sprayed onto N. excelsior and N. knightiana, which are resistant to tobacco budworms due, in part, to ovipositional nonpreference. Budworm oviposition was stimulated onto the duvane-sprayed plants both in choice experiments with conspecific unsprayed plants and with NC 2326 control plants.

(c.) The activities of several other chemicals for stimulating tobacco budworm oviposition were bioassayed. A budworm-resistant tobacco entry produced by the doubled haploid technique was used as the plant substrate for these tests. Sucrose esters containing the β-methylvaleric acid moieties (isolated from TI 165) actively stimulated budworm oviposition. Sucrose, glucose, fructose, and oxy-diols (degredation product from duvatriene diols) were all inactive.

(d.) Tobacco hornworm oviposition onto several tobacco entries was evaluated. In general, entries resistant in cage experiments are also resistant in the field. However, some field-resistant entries receive many hornworm eggs in oviposition experiments, indicating other mechanisms of resistance to tobacco hornworm exist.

3. Larval Feeding Experiments.

(a.) Plants first used in the Nicotiana species oviposition experiments were infested with tobacco budworm larvae. Each plant received five 1-2-day-old larvae, and it was then covered with a perforated plastic bag. After 7 days all surviving larvae were weighed. There was a wide-range of survival and developmental rates among Nicotiana species.

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(b.) Twenty entries were selected on the basis of insect resistance and agronomic quality from a planting at Tifton of advanced breeding lines developed from crosses of NC 82 x TI 165 and NC 2326 x TI 165. These were grown in a greenhouse under supplemental high pressure sodium lamps to stimulate the production of normal (i.e. field levels) of cuticular components. Five 1-day-old tobacco budworm larvae were placed on each plant, and survivors were weighed after one week. Field and laboratory data are being analyzed, and a budworm-resistant germplasm will be released in the near future.

B. Insect Monitoring Program.

1. A tobacco insect pest monitoring program was continued in Granville County, NC. Adult tobacco budworms, tobacco hornworms, and tomato hornworms were collected weekly in Granville County, NC from 8 traps each of 3 different types: (1) wire cone traps baited with Virelure, (2) electric grid traps baited with Virelure, and (3) blacklight traps. In addition, insect pests were monitored and collected from 20 tobacco fields throughout the growing season. Parasite numbers reared from these insects were recorded. This monitoring program has been in continuous operation since 1962. These data are useful in recognizing patterns of pest or beneficial species fluctuations. The importance of certain pest species have changed over the years, which may be correlated to changes in cultural practices of insecticide-use patterns.

2. In conjunction with our host plant resistance studies, cone-type traps, baited with a commercial preparation of Virelure that attracts male tobacco budworm moths, were monitored at Oxford, NC and Tifton, Georgia for the 4th year. Data are being combined with past and future information on Heliothis captures in order to more accurately predict peak adult population levels. These predictions will be used to better plan field experiments and for determining optimum planting dates for host plant resistance field screening tests. Similarly, data from blacklight traps at Oxford and Tifton are being used to predict hornworm outbreaks.

C. Bacillus thuringiensis studies.

1. The β-exotoxin of Bacillus thuringiensis (B.t.) var. kurstaki was tested for control of tobacco budworm larvae in spray and cornmeal bait formulations. This material did not control budworm larvae when sprayed on infested tobacco plants at rates up to 100 g/ha, but in cornmeal baits β-exotoxin was effective at the lowest rate tested (25 g/ha). There was evidence of a synergistic affect between β-exotoxin and B.t. in cornmeal baits.

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2. Six commercial formulations of Bacillus thuringiensis var. kurstaki were tested for control of tobacco budworm and tobacco hornworm larvae. Two granular formulations (Dipel 10G and Futura G) gave superior budworm control over wettable powder and liquid B.t. formulations and over an insecticide check. All materials were equally effective against hornworm larvae.

D. Cotesia (=Apanteles) congregatus Studies.

In order to determine whether parasitism of hornworm larvae by Cotesia congregatus varied depending on what part of the tobacco plant the larvae fed, laboratory-reared larvae were placed on plants in the field. Four treatments were (1) larvae on leaves 1-4 from the top, (2) leaves 5-8, (3) leaves 9-12, (4) leaves 13+. This experiment had a randomized complete block design with 6 replications (blocks) of 20-plant plots. Five larvae were put on each plant, and 30 larvae were collected from each plot one week later. The larvae were placed in 1-oz cups with artificial diet, and they were monitored periodically for two weeks. After that time all the Cotesia cocoons were counted. Over all, 72%, 84%, 69%, and 56% of the larvae were parasitized in treatments 1-4, respectively. There were ca. 40 cocoons produced per larvae, and there were no significant differences between treatments. Efforts are being made to rear these beneficial parasitoids in the laboratory.

E. Insect Rearing Activities.

A major time- and resource-consuming activity is the maintenance of laboratory colonies of tobacco budworms, tobacco hornworms, and tomato hornworms. These colonies are primarily intended for our own use, but excess insects were sent to several cooperators throughout the United States.

F. Minimum Tillage Experiments.

In a cooperative experiment with NCSU soil entomologist, Gar House, we studied the survival of tobacco budworm pupae in tobacco plots with several tillage schemes. Beneficial predators are higher in no-till and mulched plots. These insects will feed on tobacco budworm pupae. Data from this experiment are now being analyzed.

II. Graduate Students: None

III. Postdoctoral Fellows: None

IV. Publications:

Jackson, D. M. 1985. Control of tobacco insect pests with Bacillus thuringiensis var. kurstaki and β-exotoxin of B.t., 1984. Insect Acar. Tests 10:253-254.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtyk0000

Jackson, D. M. 1985. Efficacy of tank mix combinations of insecticides and sucker control chemicals on flue-cured tobacco, 1984. Insect. Acar. Tests 10:254-255.

Jackson, D. M., J. F. Chaplin, R. F. Severson, and M. G. Stephenson. 1985. Cuticular leaf chemistry and insect resistance of three reciprocally grafted tobacco types. J. Econ. Entomol. 78:815-819.

Cutler, H. G., R. F. Severson, P. D. Cole, D. M. Jackson, and A. W. Johnson. 1985. Secondary metabolies from higher plants: Their possible role as biological control agents. Amer. Chem. Soc. Symp. Ser. No. 296:178-196.

Gwynn, G. R., R. F. Severson, D. M. Jackson, and M. G. Stephenson. 1985. Inheritance of sucrose esters containing β-methylvaleric acid in tobacco. Tob. Sci. 29:79-81.

Severson, R. F., H. G. Cutler, P. D. Cole, D. M. Jackson, V. A. Sisson, A. W. Johnson, G. A. Herzog, and M. G. Stephenson. 1985. Biologically active components in the cuticulae of the Nicotiana species. Proc. 12th Annu. Meet. Plant Growth Regulator Soc. Am., July 28-Aug 1, 1985, Boulder, Colorado.

Severson, R. F., A. W. Johnson, and D. M. Jackson. 1985. Cuticular constituents of tobacco: Factors affecting their production and their role in insect and disease resistance and smoke quality. Recent Advances in Tobacco Science 11:105-174.

V. Manuscripts Accepted for Publication:

Jackson, D. M. 1986. Control of tobacco insect pests with Bacillus thuringiensis var. kurstaki formulations, 1985. Insect. Acar. Tests (in press).

Jackson, D. M. 1986. Control of tobacco budworms with β-exotoxin from Bacillus thuringiensis and with B.t. cornmeal baits, 1985. Insect. Acar. Tests (in press).

Jackson, D. M., R. F. Severson, A. W. Johnson, and G. A. Herzog. 1985. Effects of cuticular duvane diterpenes from green tobacco leaves on tobacco budworm (Lepidoptera:Noctuidae) oviposition. J. Chem. Ecol. 11: (in press).

IV. Manuscripts in Review:

Jackson, D. M., R. F. Severson, A. W. Johnson, G. R. Gwynn, J. F. Chaplin, V. A. Sisson, and G. A. Herzog. 1985. Host plant resistance in tobacco to Heliothis species. Paper presented at the Southern Regional Project S-59, "Heliothis spp: Management Systems for Field Crops", Workshop III. Emerging Control Tactics and Techniques for Heliothis. March 26, 1985. Little Rock, Arkansas.

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VII. Papers Presented at Professional Meetings:

Jackson, D. M. V. A. Sisson, and R. F. Severson. 1985. Relationship between levels of cuticular components from green leaves of Nicotiana species and oviposition by tobacco budworm and tobacco hornworm moths. Paper presented at 39th Tobacco Chemists Research Conference, Oct. 2-5, 1985, Montreal, Quebec, Abstract No. 12.

Jackson, D. M., V. A. Sisson, and R. F. Severson. 1985. Ovipositional stimulants for Heliothis virescens moths identified from Nicotiana species. Paper No. 763 presented at the 1985 National Meeting of the Entomological Society of America, Dec. 8-12, Hollywood, Florida.

Severson, R. F., K. L. McDuffie, and D. M. Jackson. 1985. The analysis of nicotine alkaloids in young green leaves of Nicotiana tabacum. Paper presented at 62nd Annual Meeting of the Georgia Academy of Science, April 26-27, 1985, West Georgia College, Carrollton, Ga. Abstract published in Ga. J. Sci. 43:19.

VIII. Graduate Student Thesis: None

IX. Acknowledgements:

I wish to thank Jimmy Cheatham, Madison Pitts, Jimmy Hobgood, and Linda Daniel for their excellent technical assistance.

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TITLE: NC03904 Soil Arthropods and Nematodes Associated with No-Tillage Practices in Tobacco

PROJECT LEADERS: G. J. House, D. P. Schmitt, and D. M. Jackson

I. Summary of Research. This project has the following objectives:

A. To investigate the impact of no-tillage and cover cropping practices on the soil arthropod and nematode community structure of tobacco.

B To compare and quantify predation on tobacco budworm prepupae and pupae occurring in the soil and on the soil surface in conventional and no-tillage systems.

C. To compare and quantify tobacco budworm survival (prepupal and pupal life-stages) under conventional and no-tillage conditions.

Soil arthropods and nematodes were quantified under four tillage treatments: (1) conventional tillage, (2) no-tillage preceded by a winter wheat cover crop, (3) conventional tillage with wheat straw mulch added in spring, and (4) no-tillage with wheat straw mulch added in the spring. All treatments were fall-ridged. The number of soil arthropods was similar in conventional and no-tillage treatments; however, there was a trend for higher numbers of soil surface-dwelling arthropods under no-tillage conditions. Furthermore, the increase in soil insects under no-tillage was ecologically balanced, as the number of predators as well as root feeders were enhanced. The combination of no-tillage and a winter cover crop provided a favorable habitat for soil-surface dwelling predaceous arthropods, especially ground beetles and spiders. The addition of wheat straw mulch had a similar effect on insect population dynamics.

Surface wheat straw was also a major factor influencing nematode populations. A trend toward higher nematode populations with straw and no-tillage was observed, but these nematodes were largely non-parasitic forms. Few plant parasitic nematodes were recovered in 1985, although there was a statistically non-significant trend for more stunt nematodes in no-tillage with straw treatments.

No major increases in tobacco budworm survival occurred under no-tillage systems. Penetration of the soil by the prepupal life-stage of the budworm may be more difficult under no-tillage than conventional tillage, which would increase the time of exposure to biotic mortality agents such as predators, and abiotic mortality factors such as body moisture loss.

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VII. Papers Presented at Professional Meetings:

Brust, G. E., G. J. House, and B. R. Stinner. 1985. Trophic role of carabid beetles in no-tillage agroecosystems. Entomological Society of America National Meeting, December 11, 1985. Hollywood, FL.

IX. We wish to thank Charles Warrick, Noele Ledford, and Jerry Brust for their excellent technical assistance.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtyk0000

Title: Biology and Control of Insects that Damage Stored Tobacco

Project Leader: Dennis W. Keever

I. Summary of Research:

This project which began in September 1985 has the following objectives:

A. To develop biological, chemical, and physical control methods and programs for the cigarette beetle in stored tobacco.

B. To determine how biotic and abiotic elements in the warehouse affect the trapping of cigarette beetles.

C. To develop models that use light trap counts to estimate insect populations in the warehouse and tobacco containers.

Four studies are either in progress or planned for the near future. The objective of the first one is to determine the freezing temperatures and the lengths of exposure to these temperatures that are required to kill all cigarette beetles within tobacco display samples. Initial results are that exposures up to 72 hours at 0, -5, and -10°C were insufficient to kill all adult beetles. At -15°C, all adult beetles were killed at exposures as low as 24 hours. No egg hatch occurred after exposures of 48 and 72 hours to -15°C and 72 hours to -10°C. Additional tests will subject greater numbers of all life stages (egg, larva, pupa, and adult) to 48- and 72-hour exposures at -15°C.

In a second stage study, tobacco warehouses have been fumigated at cool temperatures with either aluminum phosphide or magnesium phosphide. Phosphine concentrations achieved during fumigations should be sufficient to effect lower cigarette beetle populations for the coming spring, summer, and fall. Light trap counts taken both before fumigation and throughout this year will be used for efficacy assessment.

A third study will utilize pheromone traps to monitor cigarette beetles and tobacco moths in on-farm storage facilities. The objective is to determine what correlations exist between trap counts and other conditions on the farm (amount and type of commodities stored, duration of storage, type of facilities, sanitation practices, etc.). Results should aid in formulating prevention and control programs for these species.

A fourth study area is the use of Bacillus spp. as biocontrol agents of the cigarette beetle. Immediate plans are to screen various Bacillus spp. and to ascertain optimum application methods for those species that show promise. Future plans involve examining the sublethal effects of Bacillus spp. on the behavior and physiology of the cigarette beetle.

IV. Acknowledgements:

Mr. Jim Overby has used his technical skills and lengthy experience in tobacco research to greatly contribute to this project.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtyk0000

Title: N.C. 03699 Pesticide Residues in Tobacco, Tobacco Products, and Main-Stream Smoke

Project Leader: T. J. Sheets

I. Summary of Research:

Residues of fluometralin (Prime+) on Flue-Cured Tobacco and in Tobacco Soils. The 1984 experiment was established at the Border Belt Tobacco Research Station near Whiteville and at the Central Crops Research Station near Clayton. Fluometralin was applied at rates of 1.35 and 2.7 kg/ha following topping and application of a contact sucker control agent. Bottom leaf and upper leaf (tip) samples (disks from lamina) were taken immediately before and after fluometralin was applied and at 1, 2, and 4 days after fluometralin application. The green leaf disks were placed in an insulated box with solid carbon dioxide and kept frozen. Samples of cured leaf were taken from all plots, ground in a Wiley Mill to pass a screen with 20 mesh/cm, and stored at -18°C. The 1984 samples were analyzed in 1985.

In 1985, the experiments were established at the Border Belt Tobacco Research Station and at the Upper Piedmont Research Station, Reidsville. Each plot was four rows wide and 12.3 m long. Because isopropalin was an experimental variable, no herbicide was applied for general weed control. Otherwise, cultural practices and varieties were the same as those employed in 1984. Treatments in 1985 differed from those in 1984. Prior to bedding in preparation for transplanting, two plots in each replication were sprayed with 1.7 kg/ha of isopropalin as a broadcast treatment, and the herbicide was incorporated by double disking. After the tobacco was topped and a contact sucker control agent applied to all plots, fluometralin was applied as a foliar spray to one of the isopropalin plots at a rate of 1.3 kg/ha. A third plot in each block, which did not receive isopropalin, was sprayed with fluometralin at 1.3 kg/ha. A fourth plot served as an untreated control. All treatments were replicated four times at each location.

Green leaf disk samples were taken from row 2 of the control and from fluometralin treated plots at 0, 1, 2, and 4 days after application of fluometralin. Control plots were sampled before spraying on day 0. Samples were processed as in 1984. Row 3 in each plot was harvested as the leaves matured, the tobacco was cured in bulk barns, the yield recorded, and composite samples (over harvests) saved for analysis.

Wheat was planted as a cover crop at each location in October 1985, and stand counts and greens weights of seedling wheat were recorded in November (Whiteville) and December (Reidsville). Two rows of corn and two rows of tobacco will be planted in each plot in the spring of 1986 to determine injury from residue carryover in the soil.

The analytical method adopted for fluometralin residues was reliable down to a low detectable limit of about 0.01 ppm. Recoveries of fluometralin added to tobacco before extraction in amounts ranging from 0.01 to 100 ppm varied from 69 to 131%. Most recoveries fell between 80 and 110%.

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At day 0 (immediately after application), average residues on green leaves from the upper stalk position of 1.35 kg/ha fluometralin plots varied from 41.5 ppm (Whiteville, 1984) to 117.7 ppm (Reidsville, 1985). Residues of fluometralin on green lamina from the top stalk position was much higher immediately after application (day 0 values) than on low stalk samples taken at the same time. However, residues disappeared most rapidly from upper leaves, and residues on the lower leaves at day 4 after application were about equal to or greater than those on upper leaves. The average loss for the 1.35 kg/ha rate between the time of application and the day 4 sampling was 99.6% for upper stalk leaves and 93.2% for bottom stalk leaves.

This result suggested that shading by middle and upper stalk leaves protected fluometralin residues on lower leaves from photodecomposition or that some other factor was affecting residues disproportionately.

Residues on cured leaves were low for all harvests. Highest amounts were found on bottom leaves (first harvest) and lowest amounts on mid and upper leaves (third, fourth, and fifth harvests). When the residues for the 1.35 ka/ha rate on green, bottom stalk leaves at day 4 are adjusted to a 13% moisture base (as data for cured tobacco are), they become 3.04 ppm (Whiteville, 1984), 11.96 ppm (Clayton, 1984), 1.91 ppm (Whiteville, 1985), and 7.56 ppm (Reidsville, 1985). Comparison of these day 4 values for green tobacco (at 13% moisture) to corresponding values for cured tobacco shows that additional loss of fluometralin occurred between the time the day 4 samples were taken and the time of the harvest and(or) that the curing process itself reduced residues.

Counts and green weights of wheat planted in the fall of 1985 were reduced most on plots that were treated with isopropalin and fluometralin.

EBDC and ETU Residues on Flue-Cured Tobacco. Study of EBDC and ETU residues on flue-cured tobacco was continued in 1985 at the Central Crops Research Station (Clayton). Maneb and mancozeb were mixed at the recommended dilution (2 lb of formulation per 100 gal). Maneb was applied at 5-day intervals and mancozeb at 5 and 7-days beginning on May 20 (about 3 weeks after transplanting) and ending July 15, 1986. Samples of leaf were taken after curing and stored for residue analysis in the spring of 1986.

MH Residues in 1984 Flue-Cured and Burley Tobacco. MH residues in composite samples of 1984 flue-cured leaf from auction markets ranged from a low of 32 ppm to a high of 190 ppm and averaged 101 ppm over all belts and stalk positions. The average for the Old Belt was lowest (53 ppm) and that for the Georgia-Florida Belt was highest (150 ppm). Averages for the Border, Eastern, and Middle Belts were 96, 105, and 102 ppm, respectively.

In burley, MH residues were highest for Marketing Areas A and B and lowest for Marketing Area D. For the 24 composite samples, residues ranged from 14 to 89 ppm and averaged 49 ppm.

MH Residues in 1985 Flue-Cured and Burley Tobacco. Generally, MH residues in flue-cured tobacco were lower in 1985 than in 1984. In 1985, MH levels in composite samples from auction markets ranged from 11 to 178 ppm with an average of

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78 ppm. Average residues were 118 ppm for the Georgia-Florida Belt, 104 ppm for the Eastern Belt, 70 ppm for the Border Belt, 56 ppm for the Middle Belt, and 40 ppm for the Old Belt. The belt wide average (78 ppm) was the lowest one since monitoring for MH was begun in 1972. The average for 1972 was 87 ppm. Since that time, the average for every crop that was sampled exceeded 100 ppm.

The residues in 24 composite samples of 1985 burley ranged from <10 to 109 ppm. The average for all of the burley samples in 1985 (52 ppm) was nearly the same as that for 1984 burley (49 ppm).

Chlorine Content of 1983 and 1984 Flue-Cured and Burley Tobacco. The chlorine level in composite samples of flue-cured tobacco from the 1983 auction markets ranged from 0.17 to 0.44% and averaged 0.30%. For 1984, the sample range was 0.20 to 0.68% with an average of 0.41%.

The average chlorine content of burley was higher than that of flue-cured in both 1983 and 1984. The average for composite samples of burley from the four marketing areas were 0.53% in 1983 and 0.75% in 1984. The percent chlorine in three composite samples in 1984 exceeded 1.0%. Samples from Marketing Area C (Middle Tennessee and Western Kentucky) and Marketing Area D (North Carolina, Virginia, and East Tennessee) tended to have the highest levels of chlorine.

Disulfoton and Metabolites in 1984 Flue-Cured and Burley Tobacco. Composite samples of 1984 flue-cured and burley tobacco from auction markets were analyzed for disulfoton and three metabolites. The lowest detectable limits of the method of analyses were 0.06, 0.05, 0.70, and 0.10 ppm for disulfoton, disulfoton oxygen analog, disulfoton sulfoxide oxygen analog, and disulfoton sulfone, respectively. Of the 45 composite samples of flue-cured tobacco, six were positive for one of the pesticide components. Because four of the six positive values were for disulfoton barely at the lowest detectable limit and because in the same samples the values for disulfoton sulfoxide oxygen analog and disulfoton sulfone were below the lowest detectable limit, the positives for disulfoton are questionable and may be artifacts. One sample from middle stalk and one from upper stalk tobacco were positive for disulfoton sulfoxide oxygen analog (0.93 and 0.90 ppm). These were the only two samples with significant amounts of the pesticide.

Twenty-four composite samples from the 1984 burley crop were also analyzed, and none of the values exceeded the lowest detectable limits of the four compounds.

These results for burley and flue-cured tobacco indicate that US tobacco does not contain hazardous amounts of disulfoton and its toxic metabolites.

Disulfoton and Metabolites in 1984 and 1985 Tobacco Products. Samples of 20 brands of cigarettes, seven brands of cigars, seven brands of pipe tobacco, four brands of chewing tobacco, and three brands of snuff that were purchased in 1984 and 1985 were analyzed for disulfoton, disulfoton oxygen analog, disulfoton sulfoxide oxygen alalog, and disulfoton sulfone. All values for all pesticide components in all samples were below the lowest detectable limits.

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Aldicarb Residues in 1984 Flue-Cured and Burley Tobacco. An analytical method for total residues of aldicarb (aldicarb, aldicarb sulfoxide, and aldicarb sulfone) was used to analyze composite samples of flue-cured and burley tobacco from the 1984 crop. Aldicarb and aldicarb sulfoxide were oxidized to the sulfone, and the sulfone was deterined by flame-photometric gas chromatography. Four composite samples of flue cured tobacco contained total aldicarb residues above the lowest detectable limit of 0.4 ppm (two samples of bottom-stalk tobacco from the Old Belt and one middle stalk sample from the Border Belt and one from the Old Belt). The average aldicarb residue for all flue-cured samples was below the lowest detectable limit.

None of the burley samples contained measurable levels of aldicarb residues.

Effects of Simulated Residues of Four Herbicides on Flue-Cured tobacco. The four herbicides, picloram, dicamba, triclopyr, and 2,4-D, were applied to flue-cured tobacco at three locations (Central Crops, Upper Coastal Plain, and Upper Peidmont Research Stations) to simulate use of contaminated fertilizer. Rates for picloram were 0.025, 0.1, 0.4, and 1.6 g/ha; for dicamba 0.1, 0.4, 1.6, and 6.4 g/ha; for triclopyr 0.4, 1.6, 6.4, and 25.6 g/ha; and for 2,4-D 1.6, 6.4, 25.6, and 102.4 g/ha. Injury ratings in June, July, and August showed that of the four herbicides, picloram was the only one that caused significant effects. Even slight symptoms (barely discernable) of the other three herbicides was rarely seen.

Injury from picloram ranged from slight at 0.025 g/ha to severe at 1.6 g/ha. Although a rating of about 4 to 6 (based on 0 = no effect; 10 = all plants dead) was assigned to tobacco growing on plots that received 0.4 g/ha, yields were not reduced significantly. Yields, grade index, and value were reduced from application of 1.6 g/ha at all three locations.

The results indicate that, under the conditions of these tests, more than 256 times as much dicamba as picloram was needed to cause an equal degree of injury to the leaves of flue-cured tobacco. Although measurements on roots were highly variable, reductions in length, surface area, and volume of roots of picloram-treated plants appeared to be related to leaf injury and reductions in yields of cured leaf.

II. Graduate Students:

Meyer, Susan A., M. S., Toxicology
Thesis title: Effect of Application Time on Free and Bound MH in Flue-Cured tobacco

Requirements for degree completed 1985.

Atwood, Stanley T., M.S. Toxicology
Thesis title: Stability of Selected Pesticide Formulations and Combinations in Aqueous Media

Requirements for degree completed in 1985.

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IV. Publications:

Sheets, T. J., R. B. Leidy, and W. J. Mistric. 1985. Residues of methiocarb in burley and flue-cured tobacco. Tob. Sci. 29:8-11.

Sheets, T. J. and H. Seltmann, 1985. Residue and sucker control from two formulations of maleic hydrazide (MH). Beitr. Tabakforsch. 13:55-58.

Hunt, T. W., G. W. Wojeck, and T. J. Sheets. 1985. Applicator exposure to maleic hydrazide (MH) in flue-cured tobacco. Bull. Environ. Contam. Toxicol. 34:403-406.

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V. Manuscripts Accepted for Publication:

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Sheets, T. J. and B. H. Harrell, Jr. 1986. Effects of Low Levels of Soil-Spplied Picloram on Flue-Cured Tobacco. North Carolina Agri. Res. Serv. Tech. Bull. (in press).

VI. Manuscripts in Review:

Seltmann, H. and T.J. Sheets. Reapplication of MH after washoff with irrigation on sucker control and chemical residue. Tob. Sci.

Meyer, Susan A., T. J. Sheets, and H. Seltmann. MH residues in tobacco and their toxicological implications. Residue Rev.

VII. Papers Presented at Professional Meetings:

Leidy, R. B., T. J. Sheets, P. B. Shoemaker, C. E. Main, and N. T. Powell. Residues of EBDC and ETU in tobacco after application of mancozeb. Tobacco Workers Conf., Jan. 9, 1985. (Paper presented by R.B.L.).

Seltmann, H., T. J. Sheets, and C. R. Campbell. The response of bright tobacco to dicamba and 2,4-D when used as yellowing agents. Tobacco Workers Conf., Jan. 10, 1985. (Paper presented by H.S.).

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VIII. Graduate Student Thesis Completed During Reporting Period:

Effect of Application Time on Free and Bound MH in Flue-Cured Tobacco, Susan A. Meyers, Toxicology, N.C. State Univ. 1985.

Stability of Selected Pesticide Formulations and Combinations in Aqueous Media. Stanley T. Atwood, Toxicology, N.C. State Univ. 1985.

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TOBACCO GENETICS

NC 03963 GENETIC MODIFICATION OF NICOTIANA POPULATIONS

E. A. Wernsman and D. F. Matzinger

I. Summary of Research:

A. Selection Index Evaluation

Previous studies have shown that when one improves a selected trait in tobacco by genetic procedures many other characters change in an undesired direction. One example is the decrease in total alkaloids that occurs in varieties developed for higher yield. Selection index procedures are being evaluated to identify selection models that allow several traits to achieve desired levels jointly.

Black Shank Synthetic is a flue-cured tobacco population formed by intermating eight cultivars. Two types of selection have been conducted within this base population. In the first study, selection was practiced solely for increased yield by recurrent mass selection. Following 10 cycles of selection, the yield had increased by 4.23% per cycle above the checks. However, the correlated association of total alkaloids led to their reduction of 5.29% per cycle.

In the second experiment, an index was developed in an attempt to increase yield and maintain total alkaloids at the level of the base population. Following eight cycles of recurrent full-sib family selection, yield increased 2.1% per cycle and total alkaloids decreased only 0.5% per cycle. The index did restrict the rapid decrease in total alkaloids; however, the yield increase was less per cycle than when selecting for yield alone.

B. Nuclear DNA Investigations (with Lino Fragosa)

Although mitochondria contain their own genome and protein synthesizing machinery, the majority of the proteins found in the organelle are encoded in the nucleus. Import of proteins into the mitochondria is a multistep process: synthesis of a precursor on free cytoplasmic polysomes, recognition of the precursor by a receptor protein on the mitochondrial membrane, and transport of the precursor across the membrane with a concomitant proteolytic cleavage of the precursor to its mature form. There are exceptions to this pattern; some proteins are not synthesized as precursors. However, the vast majority of the proteins seem to be synthesized as precursors and their transport follows the general outline. Precursor proteins have

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a larger molecular weight than their mature counterparts due to an amino acid transit sequence found in the amino terminus of the immature protein. The transit sequence seems to contain the information required for recognition by the receptor protein. Other putative roles include transmembrane protein movement, stability and conformational changes in the tertiary structure of the protein.

We are interested in studying the informational content of the transit sequence. The approach we are taking is to isolate nuclear encoded proteins and to characterize the transit sequence by in vitro transport studies. Ultimately, gene transformation with in vitro mutagenesis will help define the parameters of the transport interactions.

A nuclear DNA library has been prepared in the bacteriophage lambda vector EMBL-3. DNA of high molecular weight isolated from leaves of tobacco was digested with the restriction endonuclease Sau3a to an average molecular size of 17 kilobases. The DNA was ligated to the BamHI site of the DNA from EMBL-3. The recombinant DNA was packaged in vitro into bacteriophage and propagated in the Escherichia coli strain P2392. The use of EMBL-3 and P2392 offers two advantages over other systems. A biochemical advantage whereby a double-digestion with the restriction endonucleases EcoRI and BamHI will render the bacteriophage DNA incapable of reforming its own structure upon ligation. The second advantage is genetical where only bacteriophage with recombinant DNA will be able to form plaques in P2392. This approach rendered a nuclear genomic library of 1.2×10^6 plaques per microgram of genomic DNA. A genomic library containing 99% of all the sequences found in N. tabacum requires only 4×10^5 plaques.

To isolate genes from this library, DNA oligomer probes were prepared from conserved regions from proteins sequenced in other organisms. Probes prepared were the beta subunit of the F_1-ATPase, aspartate aminotransferase, glutamate dehydrogenase and ribosomal protein L7. The probes were tagged with radioactive phosphorus and hybridized to plaque lifts of the genomic library. One clone isolated from the library hybridized to the oligomer of the beta subunit of F_1-ATPase. This clone was later confirmed as being the beta subunit by hybridization to a complementary DNA clone isolated from N. plumbaginifolia. The aspartate aminotransferase and ribosomal protein L7 probes hybridized to one and four plaques, respectively.

As a second approach, complementary DNA was prepared from cytoplasmic mRNA and cloned into the vector lambda gt11. This vector allows the synthesis of the protein encoded by the complementary DNA sequence. However, this vector does not allow for a biochemical or genetical selection of the recombinant

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molecules. We are generating a second cDNA library in a derivative of lambda gt11 which contains a biochemical selection for recombinant molecules.

Antibodies are being generated for a mixture of mitochondrial proteins which will be used to select clones in the lambda gt11 expression library. To identify the protein encoded by the cDNA, the sequence will be compared to that of known proteins from other systems. Due to the fact that approximately 200-300 mitochondrial proteins are encoded in the nucleus, this approach should provide cDNA clones for various proteins.

C. Mitochondrial DNA Genes (with M. M. Bland)

All mitochondria encode proteins involved in respiratory chain function. These proteins include subunits I, II, and III of the cytochrome c oxidase complex, subunit 6 of the ATPase complex and cytochrome b of the bc_1 complex. A few mitochondrial proteins are encoded by the mitochondrial genome in some organisms, but in others, they are encoded by nuclear genes. For example, F_0-ATPase subunit 9 is a nuclear gene product in mammals, but is encoded by mtDNA in yeast and higher plants. Neurospora may contain both nuclear and mitochondrial copies of the subunit 9 gene.

The mtDNA of yeast and Neurospora encodes a single small subunit ribosomal protein. Other ribosomal proteins are encoded in the nucleus, synthesized on cytosolic ribosomes, and imported into the mitochondria for assembly. In mammals there is no evidence that any ribosomal proteins are encoded in the mitochondria.

We have isolated an actively transcribed segment of mitochondrial DNA from tobacco that contains the F_0-ATPase subunit 9 gene, an open reading frame with homology to the E. coli small subunit ribosomal protein S13 and an open reading frame with homology to a portion of the mammalian "URF 1" protein, recently shown to be a component of the NADH:ubiquinone reductase complex (NADH:Q 1). The transcription patterns of the tobacco ATPase 9 gene and S13-like open reading frame share eight RNA bands indicating the two sequences are part of the same transcriptional unit. A maize mtDNA fragment contains the S13 homologous sequence and the NADH:Q 1 homologous sequence in an orientation similar to tobacco. The S13-like sequence is present as a single copy in maize and tobacco, as two copies in wheat, and is absent in pea and bean.

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D. Anther Culture Induced Genetic Variation and its Value in Tobacco Improvement

In vitro anther culture of near homozygous tobacco (Nicotiana tabacum L.) cultivars yields haploids which, when chromosome doubled, result in populations of doubled-haploid (DH) lines exhibiting great among-line genetic variability with agronomic characteristics inferior to those of the cultivars. Studies were conducted to characterize the nature of this "induced" genetic variability and to determine its potential value in recurrent selection programs. Sixty-four DH lines from a single plant of cultivar 'NC 95' were randomly assigned to eight sets of a North Carolina Design II mating design. DH full-sib families and selfed progenies of the parental plant were evaluated for seven variables in two field environments. Genetic variances in the DH population were estimated, heritabilities calculated, and genetic gains from practicing half-sib and full-sib family selection for yield improvement in the DH population were predicted. A DH population composite, a random-mated DH population, and populations resulting from practicing half-sib and full-sib family selection in the DH population were compared to two sublines of the original parental plant. Significant additive genetic variance was detected for six of seven variables measured in the DH population. Dominance variance estimates were zero or negligible. Random mating of DH lines resulted in a recovery of 26% of the yield loss of the DH population composite compared to the cultivar. One cycle of half-sib and full-sib family selection resulted in populations yielding 5% and 6%, respectively, more cured leaf than the random-mated DH population; both selected populations were lower yielding than the sublines of the original parental plant. Two or more cycles of recurrent full-sib selection are estimated to be required to achieve a population equal in yielding ability to the parental cultivar. This requirement reduces the appeal of androgenic somaclonal variability as a germplasm source for tobacco improvement.

E. Induction of a Virus Resistant Mutant Through Anther Culture

Potato virus Y strain NC 78 (strain III) produces a severe necrotic reaction on all American flue-cured cultivars. Small plants growing at cool temperatures (less than 22 degrees) are usually killed by the virus within three weeks of inoculation. Hence, the disease reaction of this germplasm and this virus strain is highly qualitative. Highly susceptible flue-cured cultivar McNair 944 was subjected to in vitro anther culture and 545 haploid plants of a 5-7 cm diameter size were inoculated with PVY strain 78. Five hundred forty-four plants were susceptible and severely stunted or killed by the virus; one

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plant was resistant. Back inoculation of Burley 21 plants with inoculum prepared from leaves of the resistant haploid revealed the presence of virus in the plant, and gel-diffusion tests with PVY antisera confirmed the virus to be PVY. Cytological examination of the resistant plant confirmed the presence of 24 chromosomes and aberrations were not evident.

Leaf midvein of the resistant plant was subjected to *in vitro* tissue culture to achieve chromosome doubling and doubled haploid (2n=48) plants were produced. These plants were self pollinated and hybridized reciprocally with parental cultivar McNair 944.

McNair 944, the resistant doubled haploid, DH 602, and the F_1 hybrids were grown in five replications of a randomized complete blocks design in the greenhouse. In each plot, 13 plants were inoculated with strain 78 and one plant served as an uninoculated control. Results are shown in Table 1.

Table 1. Mean reaction of McNair 944, DH 602, and their F_1 hybrids to potato virus Y, strain 78.

	Disease Symptoms			
Genotype	Lamina Necrosis	Veinal Necrosis	Stunting	Mosaic
	% disease			
McNair 944	Dead	Dead	Dead	Dead
DH 602	1	0	28	22
DH 602 x McNair 944	63	19	31	85
McNair 944 x DH 602	69	22	36	77

Although the virus produced some stunting and a mild mosaic reaction, plants of DH 602 were highly resistant. The F_1 and reciprocal F_1 hybrids did not differ, and their disease reactions were intermediate between the two parents. The hybrids suffered a severe initial shock upon inoculation, but later recovered considerably. These data demonstrate that the genetic mechanism controlling the disease reaction is nuclear, and the gene(s) responsible exhibit additive or codominant effects. The anther culture-induced mutant appears to be a promising source of PVY resistance, but additional information on reaction to other PVY strains, other viruses, and its mode of inheritance is needed.

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II. Graduate Students:

Bland, Molly M., Ph.D. Candidate, Genetics. Molecular genetics of tobacco.

Corbin, Thomas C., Ph.D. Candidate, Crop Science. A comprehensive tobacco improvement program utilizing parthenogenic haploids.

Witherspoon, William David, Jr., Ph.D. Candidate, Crop Science. Utilization of the haploid sporophyte as the selection unit in plant breeding.

Yung, Carl H., Ph.D. Candidate, Crop Science. DNA amplification in anther culture-derived haploids and its relationship to changes in tobacco metabolism.

III. Postdoctoral Fellows:

Fragosa, Lino. Cloning of Nicotiana DNA.

IV. Publications:

Bland, M. M., D. F. Matzinger, and C. S. Levings, III. 1985. Comparison of the mitochondrial genome of Nicotiana tabacum with its progenitor species. Theor. Appl. Genet. 69: 535-541.

Bowman, D. T., E. A. Wernsman, T. C. Corbin, and A. G. Tart. 1985. Performance of four Maryland tobacco cultivars under two different harvest schemes in North Carolina. Tob. Sci. 29: 16-19.

Cockerham, C. C. and D. F. Matzinger. 1985. Selection response based on selfed progenies. Crop Sci. 25: 483-488.

Gooding, G. V. Jr, E. A. Wernsman, and R. C. Rufty. 1985. Reaction of Nicotiana tabacum L. cultivar Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus and Peronospora tabacina. Tob. Sci. 29: 32-35.

Schnell, R. J. and E. A. Wernsman. 1986. Androgenic somaclonal variation in tobacco and estimation of its value as a source of novel genetic variability. Crop Sci. 26: 84-88.

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V. Manuscripts in Review:

Bland, M. M., C. S. Levings, III, and D. F. Matzinger. The tobacco mitochondrial ATPase subunit 9 gene is closely linked to an open reading frame for a ribosomal protein. (In Review).

Bowman, D. T., E. A. Wernsman, T. C. Corbin, and A. G. Tart. Stability of flue-cured tobacco cultivars for yield and quality. (Tobacco Science).

Rufty, Rebeca C., E. A. Wernsman, and G. V. Gooding, Jr. Evaluation of tobacco haploids and doubled haploids for resistance to tobacco mosaic virus, Meloidogyne incognita and Pseudomonas syringae pv. tabaci using detached leaves. (Phytopathology).

Rufty, Rebeca C., E. A. Wernsman, and G. V. Gooding, Jr. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar Havana 307. (In Review).

Wernsman, E. A. and R. C. Rufty. Tobacco. In Handbook of Plant Breeding II, W. F. Fehr (ed.) Macmillan Publishing Co. Inc. New York, NY.

VI. Papers Presented at Professional Meetings:

Corbin, T. C. and E. A. Wernsman. Comparisons of genetic gains from maternal dihaploid, S_1, and FS Recurrent Family Selection. Amer. Soc. Agron. Annual Meeting, Dec. 1-6, 1985, Chicago, IL (Agron. Abstr. p.50. 1985).

Rufty, R. C., E. A. Wernsman and G. V. Gooding, Jr. Evaluation of tobacco haploids for resistance to TMV, PVY and Meloidogyne incognita using detached leaves. Amer. Phytopathology Soc. Annual Meeting, Aug. 11-15, 1985. Reno, NV.

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Title: NC 05590 Characterization of cis-acting sequence determinants for the regulation of gene expression in plants

Project Leader: Mark A. Conkling

I. Summary of Research:

The objective of this research program is to characterize DNA sequences which act in cis to regulate organ-specific gene expression in tobacco. To do this we are cloning genes which are expressed in organ-specific fashions. These will be reintroduced into tobacco cells. Following plant regeneration, the organ-specific expression of the input gene will be determined. Those genes which exhibit the predicted organ-specific expression must contain sequences regulating that expression and will be characterized further.

Before arriving in North Carolina, I had isolated a collection of cDNA clones which are homologous to tobacco mRNA's which exhibit organ-specific expression. These mRNA's are expressed specifically in either leaves or roots, are expressed at high levels, are transcriptionally regulated, and are members of small gene families. These cDNA clones have been used to screen a tobacco genomic library and the corresponding genes have been isolated. These are being characterized to determine the regions of the cloned genomic fragments which are transcribed. The technologies for transforming tobacco cells using Agrobacterium vectors have been established in the laboratory.

VII. Papers Presented at Professional Meetings:

Conkling, M. A., Cheng, C. L., and Goodman, H. M. (1985) Isolation and characterization of organ-specific cDNA clones from tobacco. UCLA Symposia on Molecular and Cellular Biology.

Conkling, M. A., Cheng, C. L., Pfitzner, U., and Goodman, H. M. (1985) Isolation and characterization of organ-specific cDNA clones from tobacco. First International Congress of Plant Molecular Biology.

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Title: NC03896 Cell Culture Techniques for the Development of Disease Resistance in Tobacco

Project Leader: Margaret E. Daub

I. Summary of Research:

This project has three broad objectives:

1. To investigate the potential of somaclonal variation for generating novel, disease-resistant genotypes.

2. To develop protoplast fusion techniques for transferring naturally occurring resistance genes from wild Nicotiana species into tobacco cultivars.

3. To investigate the molecular mechanism of resistance in one host-pathogen system (the tobacco/Cercospora nicotianae system) in order to identify genes for resistance which may be important for tobacco improvement.

A. Somaclonal Variation. One of the most promising areas in the use of tissue culture for the development of novel crop genotypes is the observation that the process of growing cells in culture can generate stable, heritable variation which is useful in crop improvement. This variation has been termed somaclonal variation, and has been demonstrated in a number of crops including sugar cane, potatoes, and tomatoes, and for a number of different traits including both disease resistance and agronomic traits. The purpose of this work is to investigate the usefulness of somaclonal variation for developing high quality tobacco cultivars with increased levels of disease resistance.

We have isolated mesophyll protoplasts from two tobacco cultivars (Coker 319 and NC 2326) which have desirable quality characteristics, but carry no resistance to Tobacco Mosaic Virus (TMV) and root knot nematodes, and only low levels of resistance to black shank. The protoplasts were induced to form callus, and were maintained as callus for several months in order to increase the probability of inducing variability. Plants were then regenerated from these calli, and transferred to soil in the greenhouse. These plants were allowed to flower, and selfed seed was collected from each plant. This latter step is required in order to ensure that all variation seen among the plants is limited to changes which are passed through a seed cycle.

Over 2500 seed progeny of 64 somaclones were tested in 1985 for black shank, TMV, and root knot nematode resistance. Black shank resistance tests were conducted both in the greenhouse and in disease nurseries at the Upper and Lower Coastal Plain Research Stations. TMV and root knot nematode resistance was determined in greenhouse tests. Thus far we have identified three somaclones whose progeny showed elevated levels of black

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shank resistance in both field and greenhouse tests. We have found no increase in TMV or root knot nematode resistance. In 1986 we will again test the three black shank resistant somaclones, as well as progeny of individual selections made from these clones in the field in 1985. We will also be testing approximately 6000 progeny from 75 new somaclones in the field and greenhouse for resistance to all three pathogens.

In the future we are hoping to use this technique to develop lines with high levels of resistance to Granville wilt. Results from other laboratories (and our own preliminary results) indicate that highly resistant lines are most likely to be derived from parents which have a low level of resistance initially; resistant lines often cannot be derived from highly susceptible parents. We are thus isolating somaclones from two varieties, K 399 and Speight G-28, which carry resistance to Granville wilt, but which do not have as high a level of resistance as would be desirable. To test these lines we are working to establish a Granville wilt nursery at the Lower Coastal Plain Research Station.

B. Transfer of Resistance Genes from Nicotiana repanda to Nicotiana tabacum by Protoplast Fusion. The wild species, Nicotiana repanda, has been shown to possess resistance to more tobacco diseases than any other Nicotiana species. Its resistance to three root knot nematode species and to TMV are of particular importance for tobacco improvement. Attempts to produce hybrids of N. repanda and N. tabacum by conventional breeding methods have been unsuccessful. To overcome the obstacles to sexual hybridization, somatic hybridization was attempted by fusion of protoplasts and culturing of somatic cells.

In order to identify any somatic hybrids produced by protoplast fusion, we used protoplasts of N. tabacum plants homozygous for the semi-dominant nuclear albino mutation (Su/Su). Since our N. repanda plants lacked this gene, we could select for hybrid fusions by looking for light green shoots in contrast to the albino and dark green shoots of the parents. The leaves of albino plants grown in culture at 25°C in continuous light proved a good source of protoplasts. Yields of leaf mesophyll protoplasts from N. repanda plants grown in the greenhouse were unreliable, especially during the winter months. After a series of tests using plants grown under different conditions in the Phytotron, optimum growing conditions for the production of protoplasts were found to be day/night temperatures of 30/26°C and 9 hours of illumination.

Protoplasts of the two species were released by overnight incubation in 1% Cellulysin and 0.2% Macerase, dissolved in protoplast buffer with 0.5 M mannitol. After isolation the protoplasts of the two species were mixed and fused by the addition of polyethylene glycol (PEG) (MW 8000). After washing

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out the PEG, the cells were cultured in liquid medium with decreasing concentrations of mannitol for one month, then cell clumps were transferred to regeneration medium without mannitol. When shoots were produced, albino and dark green regenerants were discarded and light green ones transferred to rooting medium. When light green plants were 2-3" high and had a good root system they were transferred to pots in the greenhouse. Optimum growing conditions for these plants proved to be in a greenhouse at 80°C, shaded with brown paper and watered sparingly. Even under these conditions some light green plants did not survive.

Out of 50 fusions using PEG, plants were regenerated from only 5, probably due to the phytotoxicity of PEG. These poor results prompted our attempts at electrofusion. In electrofusion, protoplasts are aligned between electrodes in a field of alternating current, then fused by short pulses of direct current. Protocols were established for fusing both green N. repanda and albino N. tabacum protoplasts with like protoplasts but no protocol was found for fusing unlike protoplasts. Voltages and pulse durations that caused albino protoplasts to fuse had no visible effect on the green protoplasts; whereas those which fused the green protoplasts completely disrupted the albino protoplasts.

At present, of the 17 light green plants that have been examined cytologically, ten had chromosome numbers between 46 and 48 and are thought to be N. tabacum, and seven had chromosome numbers between 87 and 95 and are thought to be N. tabacum + N. repanda hybrids. Most of the plants with the higher chromosome numbers have varying leaf shapes, puckered leaves, a rosette-type growth habit and dark green areas on their leaves, indicating that chromosome loss and rearrangement are continuing. None of the light green regenerants has flowered.

Peroxidase and esterase isozymes of the parents and putative somatic hybrids are to be compared to confirm the hybrid nature of the light green plants regenerated. Disease resistance tests and fertility tests of the hybrids will also be done to determine their usefulness in a tobacco breeding program.

We are currently working to develop methods which will improve and simplify protoplast fusion protocols. Although the albino selection system has worked well for us, there are several major problems associated with this system. First, the Su gene, originally isolated in a broad leaf tobacco cultivar, must be crossed into the desired flue-cured cultivars. This process is very time consuming. Second, the fusion hybrids cannot be identified until plants are regenerated from the cultures. This means that we must make monthly transfers of cultures derived from all of the protoplasts (several thousand for each fusion experiment) for 6-8 months until regeneration occurs and we can identify the few that are hybrids. Finally, the presence of the Su gene in the hybrids results in a severe reduction in the

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amount of chlorophyll present. This further weakens our already weak hybrids, and many of them do not survive transfer to soil. Thus there is a major need for alternative selection methods.

We have devised a method which we feel will revolutionize current protoplast fusion protocols and make them almost routine for hybridizing tobacco and many other crop species. We have recently obtained two transformation vectors from Ciba-Geigy Corporation. These vectors, specially constructed strains of Agrobacterium tumefaciens, genetically transform plants for resistance to two different antibiotics, kanamycin and hygromycin. Thus we can transform N. repanda with one of the vectors and any desired flue cured cultivar with the other. Following protoplast isolation and fusion, we can plate the protoplasts on a medium containing both antibiotics, and only the fusion hybrids (which will have resistance to both antibiotics) will surive. We can thus overcome all of the problems faced with our albino selection system. We feel that the results of our studies will be to provide a versatile antibiotic selection protocol. This will aid the development of new cultivars not only of tobacco, but also of many other crops, for these vectors can transform any dicotyledonous species.

C. Mechanisms of Resistance to the Phytotoxin Cercosporin Produced by Members of the Genus Cercospora. Cercospora species are a group of plant pathogenic fungi which cause serious losses worldwide on a large number of host species including tobacco, banana, sugar beet, peanut, coffee, rice, and corn. Cercospora species are an interesting group of plant pathogens, not only because of the vast number and diversity of hosts which they attack, but also because they have been very hard to control due to the difficulty in identifying adequate levels of resistance in natural populations of host plants. One reason for the success of this group of pathogens appears to be their ability to produce the extremely toxic, free-radical-generating phytotoxin, cercosporin.

Cercosporin is a photosensitizing compound which is activated by light and then reacts with O_2 to produce singlet oxygen and superoxide. In the plant cell, cercosporin damages the cell membranes by causing a peroxidation of the membrane lipids. The resulting changes in the structure and composition of the plant cell membranes make them very leaky, and it is hypothesized that this provides the pathogen (which is limited to growth within the intercellular spaces of the leaf) with the nutrients required for growth and sporulation in the host. At this time, no sources of resistance to cercosporin in plants have been identified. All plants which have been tested (including those which carry some resistance to the pathogen) are sensitive to cercosporin, and we have been unable to select cercosporin-resistant tobacco and sugar beet cells or protoplasts in culture. Mice and bacteria are also sensitive to cercosporin. The fungus Cercospora and a large number of other fungi, however, are highly

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resistant to cercosporin and to another singlet oxygen-generating sensitizer, hematoporphyrin. Considering the extreme toxicity of singlet oxygen, the generalized resistance of fungi is surprising, and gives us a unique opportunity to investigate resistance mechanisms, not only to this very damaging phytotoxin, but also to free-radical-generating agents in general.

The purpose of this work is to determine the genetic and biochemical basis of resistance to cercosporin. Since we have not been able to identify or induce cercosporin-resistance in plants, we are using the naturally-resistant fungus as our test system. At the present time, we have determined that antioxidants and oxidative enzymes such as superoxide dismutase do not play a significant role in resistance; levels of antioxidants and of superoxide dismutase do not differ significantly between Cercospora species and the cercosporin-sensitive fungus, Phytophthora cinnamomi. The presence of carotenoids (which are very effective quenches of singlet oxygen) and the compositionof the fungal cell wall do, however, appear to play a role in resistance.

We have determined that Cercospora nicotianae and other Cercospora species produce high concentrations (approximately 20 µg/gram dry weight) of carotenoids at early stages of cell growth. β-carotene, a potent singlet oxygen quencher, accounts for approximately 99% of the carotenoids present. Further, mutants of two other fungi, Neurospora crassa and Phycomyces blakesleeanus which are blocked in carotenoid production are considerably more sensitive to cercosporin than wild type isolates. It is interesting to note that wild type isolates of Neurospora crassa produce the same amount of carotenoids as do Cercospora species but are considerably more sensitive to cercosporin, showing about 80% inhibition when grown on media containing 10 µM cercosporin. These results suggest that carotenoids are an important factor in cercosporin resistance, but they clearly are not the sole basis of resistance.

We also have evidence that the fungal cell wall plays a role in resistance, perhaps by acting as a barrier to cercosporin penetration. We have developed methods for isolating protoplasts from species of Cercospora. These protoplasts are considerably more sensitive to cercosporin than is the fungal mycelium. Furthermore,the protoplasts rapidly regain resistance to cercosporin as they begin to regenerate a cell wall. For example, freshly isolated C. nicotianae protoplasts are all killed by exposure to 10 µM cercosporin (a concentration which is not toxic to mycelium). By 12 hrs., however, over 50% of the protoplasts have become resistant to cercosporin. We have determined that during this 12 hr. period, cell wall regeneration is occurring as evidenced by a gradual resumption of osmotic resistance by the protoplasts, and the binding of fluorescent compounds which specifically bind to fungal cell wall constituents. Our strongest evidence for a role of the cell wall

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comes with experiments with protoplasts of Neurospora crassa. As stated earlier N. crassa mycelium is quite sensitive to cercosporin, even though the fungus produces high concentrations of carotenoids. We have found that N. crassa protoplasts show the same level of sensitivity to cercosporin as do Cercospora protoplasts, but as the Neurospora protoplasts start to regenerate, they do not become any more resistant. Thus a significant proportion of cercosporin resistance appears to reside in the Cercospora cell wall. Our current research is directed at analyzing the cell walls of Cercospora species and of N. crassa in order to try and determine what the components are that are responsible for resistance.

It is hoped that this information will be useful in developing new and better ways of controlling diseases caused by Cercospora species, and perhaps other plant pathogenic fungi as well. If we are in fact unable to induce resistance to the toxin in the host plant, perhaps we can approach the problem from an opposite, but equally effective angle, that is, trying to disrupt the resistance mechanisms of the fungus itself. This could be accomplished by the use of new, non-toxic chemical control which is targeted at disrupting a resistance mechanism rather than killing the fungus. Alternatively, it may be possible to selectively breed for compounds in the host which disrupt the fungal defense mechanisms. The possibility that plants can be genetically modified, not to resist pathogens, but to disrupt their virulence mechanisms is an important area that needs to be pursued.

II. Graduate Student:

Kimberly D. Gwinn, PhD Candidate in Plant Pathology. Thesis Topic: "Resistance to the Photosensitizing Fungal Toxin Cercosporin".

III. Postdoctoral Fellow:

Dr. Anne E. Jenns. Research Area: Protoplast Fusion in Tobacco.

V. Manuscripts Accepted for Publication:

Daub, M. E. 1986. tissue culture and the selection of resistance to pathogens. Ann. Rev. Phytopathol. 24 (In Press).

VI. Manuscripts in Review:

Gwinn, K. D. and Daub, M. E. Protoplasts from Cercospora species - isolation and reaction to the phytotoxin cercosporin. (Submitted to Experimental Mycology).

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VIII. Papers Presented at Professional Meetings:

Daub, M. E. and Payne, G. A. 1985. The role of carotenoids in resistance of fungi to cercosporin. Phytopathology 75:1298 (Abstr.).

Gwinn, K. D. and Daub, M. E. 1985. Relationship of fungal cell wall regeneration to cercosporin resistance. Phytopathology 75:1298 (Abstr.).

IX. Acknowledgements:

The excellent technical assistance of Mr. D. Wayne Pollard is greatly appreciated. This work has been supported by grants from R. J. Reynolds Corporation, The North Carolina Tobacco Foundation, and the USDA Competitive Grants Program.

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PLANT PATHOLOGY

STATE 3572: EPIDEMIOLOGY AND CONTROL OF VIRUS DISEASES OF SOLANACEOUS CROPS

G. V. Gooding, Jr., Project Leader

I. Summary of Research

A survey was conducted in 1984 to establish the identity, distribution, and relative importance of viruses in burley tobacco. Tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV) were of high incidence in the crop and considered causing enough losses to justify breeding for resistance to them. Randomly collected isolates of TVMV (± 50) and TEV (± 40) from the 1984 survey were assayed in 1985 to determine the strain composition of the population. The reaction of the different isolates was determined on different tobacco germplasm to characterize the different isolates. Germplasm consisted of commercial cultivars Ky 14, B49, Ky 14XL8, and virus-resistant sources Greenville 107, Ha 307, and V20. Based on the reaction of these hosts to the different isolates, TVMV consisted of a single strain but TEV of a number of strains varying from mild to severe in their effect on the different germplasm. A type isolate of TEV was selected representing a mild strain, a moderate strain, and a severe strain.

Evaluation of commercial cultivars of burley tobacco and virus-resistant tobacco germplasm for their reaction to TVMV and TEV in field tests was completed. Field evaluations were conducted at three locations in North Carolina and one location in Tennessee in 1984 and 1985 in cooperation with Drs. R. C. Rufty and Robert Miller.

Cooperation was continued with plant breeders to develop resistance to potato virus Y (PVY) in flue-cured tobacco.

A mild strain of tobacco mosaic virus (TMV) significantly reduced losses in tobacco to the common strain (cross protection) in field trials conducted at the Oxford Research Station in cooperation with Dr. Harvey Spurr.

A mild strain of PVY from potatoes in the United States protected tobacco from a necrotic strain from Chile by preventing infection by the necrotic strain. The protection mechanism in this system is, therefore, prevention of infection by the severe strain rather than interference with the adverse effects of the severe strain in the host.

An evaluation of the control recommendations for tobacco mosaic virus was continued on the Dennis Smith farm in Duplin County. Incidence of TMV was approximately 50% on 60 acres of tobacco in 1983. Incidence was approximately 20% on 6 acres of tobacco in 1985 and 90% of the TMV was on two fields where tobacco followed tobacco. Experience on

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this farm indicates that TMV can be effectively controlled if the recommendations are implemented.

II. Postdoctoral Fellow:

Dr. E. K. Park completed a year conducting research on potato virus Y. His research was focused on resistance and biological and biochemical characterization of strains of PVY.

III. Publications:

Gooding, G. V., Jr. 1985. Relationship between strains of potato virus Y and breeding for resistance, cross protection, and interference. Tobacco Science 29:99-104.

Gooding, G. V., Jr., and Kennedy, G. G. 1985. Resistance in tobacco breeding line NC 744 to potato virus Y and inoculation by aphids. Plant Dis. 69:396-397.

Gooding, G. V., Jr., Wernsman, E. A., and Rufty, R. C. 1985. Reaction of Nicotiana tabacum L. cultivar Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus, and Peronospora tabacina. Tobacco Science 29:32-35.

Gooding, G. V., Jr., and Moyer, J. W. 1985. Host index of plant viruses in North Carolina. pp. 5-122 IN: L. F. Grand (ed.) North Carolina Plant Disease Index. NC Agr. Res. Serv. Tech. Bull. 240 (Rev.). 157 p.

Averre, C. W., and Gooding, G. V., Jr. 1985. Virus diseases of tomato and their control. Plant Path. Inf. Note 186.

IV. Manuscripts Accepted for Publication:

Gooding, G. V., Jr. Tobacco mosaic virus. IN: The Rod-Shaped Viruses. Van Regenmortel and Frankel-Conrat, eds. Plenum Publishing Corp., New York.

Miner, G. S., Gooding, G. V., Jr., and Moore, J. M. Control of inoculum sources of tobacco mosaic virus on tobacco plant beds. Tobacco Science.

V. Manuscripts in Review:

Gooding, G. V., Jr., and Rufty, R. C. Distribution, incidence, and strains of viruses in burley tobacco in North Carolina. Plant Disease.

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Park, E. K., and Gooding, G. V., Jr. Tolerance of Nicotiana tabacum cultivars Dixie Bright 244-2, McNair 30, and Golden Stock Penish to strains of potato virus Y. Plant Disease.

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Evaluation of tobacco haploids and double-haploids for resistance to tobacco mosaic virus, Meloidogyne incognita, and Pseudomonas syringae pv. tabaci using detached leaves. Phytopathology.

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar Havana 307. Phytopathology.

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Title: NC 03773 Soilborne Diseases of Tobacco

Project Leader: H. D. Shew

I. Summary of Research

Tests were conducted to determine how sensitivity to the fungicide metalaxyl is inherited through the asexual reproductive cycle. First and second generation zoospores of Phytophthora parasitica var. nicotianae (P.p.n.) were collected and screened on metalaxyl amended agar. Zoospores of sensitive parents were always sensitive (ED_{50} > 0.1 ppm) whereas 200 spores of moderately sensitive parents segregated into sensitive and moderately sensitive progeny. These results would indicate that sensitivity to the fungicide is a recessive trait. Further studies will attempt to determine the percentage of nuclei in a population of isolates that are resistant to the fungicide.

A series of experiments was conducted on the Rhizoctonia leaf spot disease of flue cured tobacco. The effect of temperature and misting regime on production of basidiospores and subsequent infection of tobacco were determined in phytotron chambers. Optimum temperatures for reproduction and infection are 22 to 28 C. Little reproduction and infection occur below 22 C and above 28 C. Leaf wetness was necessary for infection to occur. Percent leaf area damage increased as duration of misting increased. Typical % leaf area damage was 1, 15, 24, and 46% with no misting, night misting once every 20 min., night misting once every 5 min., and 24 hr misting once every 5 min., respectively. Basidiospores were trapped and were found to be present in very high numbers during day and night. Peak release occurred in early morning, mid-afternoon, early evening. A host range study has been conducted and several hosts in addition to tobacco have been identified, including eggplant and sugarbeet. Sensitivity of the foliar Rhizoctonia isolates to the fungicide Benomyl was determined and compared to stem rot isolates of Rhizoctonia. The foliar isolates have a lower sensitivity to benomyl but are not considered tolerant. Morphology, fungicide sensitivity and growth rate indicate that the foliar isolates of Rhizoctonia are different than tobacco sore shin isolates of Rhizoctonia. Current research is addressing the question of origin of the foliar isolates and why the disease has caused significant losses the last 2 years. Additional information is presented in tables 1-3.

A cooperative project was continued with Drs. Echandi and Powell on the effectiveness of fall fumigation in conjunction with the use of plastic mulching on the control of Granville wilt of tobacco. As in 1984, excellent control was obtained with the use of plastic plus fumigant.

A joint research project with Dr. C. E. Main was continued on loss assessment evaluation with the tobacco black shank disease.

A survey was conducted in cooperation with Dr. P. B. Shoemaker on the level of the black root rot fungus in burley tobacco soils.

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Thirteen problem fields in five counties were sampled prior to transplanting and assayed for the pathogen. Pathogen populations were correlated with root rot severity determinations on a susceptible cultivar planted in the problem fields.

Table 1. Effect of temperature regime on hymenial formation by Thanatephorus cucumeris in soil[x]

Temperature (C)	% Positive
22-16	69
26-20	93
30-24	83
34-28	17

Table 2. Effect of misting regime on hymenial formation by Thanatephorus cucumeris in soil

	% Positive/isolate		
Misting regime	1600	1602	1609
None	0	31	22
Night Only (3x/hr)	39	86	77
Night Only (12x/hr)	77	92	77
Day + Night (12x/hr)	72	83	88

Table 3. Effect of misting regime on leaf area damage by Thanatephorus cucumeris in soil

Misting regime	% Leaf Area Damage
None	1.4
Night Only (3x/hr)	15.0
Night Only (12x/hr)	23.5
Day + Night (12x/hr)	46.0

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A set of differential burley cultivars were selected and are being screened for black root rot resitance using pathogen isolates collected from different counties. The objective of this cooperative project with Dr. Rufty of Crop Science is to determine if races of Thielaviopsis basicola exist in order to develop a method of screening for resistance for black root rot on new cultivars.

II. Graduate Students

R. J. Pulliam, M. S., Plant Pathology. "Infection and Colonization of Susceptible and Resistant Flue-Cured Tobacco by Phytophthora parasitica var. nicotianae.

IV. Publications

Shew, H. D. 1985. Response of Phytophthora parasitica var. nicotianae to metalaxyl exposure. Plant Disease 69:559-562.

Shew, H. D., and Main, C. E. 1985. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina. Plant Disease 69:901-903.

Sidebottom, J. R. and Shew, H. D. 1985. Interaction of soil type and soil matric potential on infection of tobacco by Phytophthora parasitica var. nicotianae. Phytopathology 75:(1435-1438).

Sidebottom, J. R. and Shew, H. D. 1985. Effects of soil texture and matric potential on sporangium production by Phytophthora parasitica var. nicotianae. Phytopathology 75:(1439-1443).

VII. Papers Presented at Professional Meetings

Shew, H. D. and C. E. Main. 1985. Leaf spot of tobacco caused by Rhizoctonia solani. Phytopathology 75:(1278).

IX. I gratefully acknowledge the technical assistance of D. T. Glover, E. C. Gray, and S. Bhikhai in this study.

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Title: NC 09326 Effects of Water Stress on Expression of Host Resistance to a Phytophthora sp.

Project Leader: H. D. Shew

I. Summary of Research

Research during this period concentrated on two areas. First, the effects of soil type, soil matric potential, and different levels of quantitatively inherited host resistance on inoculum production by the soilborne pathogen Phytophthora parasitica var. nicotianae were determined. Four tobacco cultivars ranging in resistance from none to high (based on field response to P.p. var. nicotianae) were inoculated with a standardized zoospore suspension and then incubated in 2 conducive soils (previously determined) maintained at 3 matric potentials. Inoculum production was determined on selective media after 4 and 8 wks incubation. All treatments significantly affected inoculum production. Inoculum production per milligram root (fresh weight) was 4.1, 3.4, 2.8, and 2.6 for the susceptible, low, moderate, and high resistance cultivars. A highly significant interaction occurred between soil type and matric potential regime. The lowest inoculum production was observed at -0.1 bars in both soils and greatest at -1.0 and -10.0 bars. Inoculum production was greater in a highly conducive silt loam soil than in a sandy loam soil which contained organisms antagonistic to P.p. var. nicotianae. In a field study propagules of P.p. var. nicotianae per gram of soil from the root zone of symptomatic plants were 43, 30, 16, and 5 for the susceptible, low moderate, and high resistance cultivars, respectively. No differences were observed in infection and initial colonization of the 4 cultivars using whole mounts and thin sections. Additional information on the effects of resistance, soil type, and matric potential is presented in tables 1-3.

The second area of research dealt with the effect of water stress and host resistance on development of tobacco black shank. A microplot test was conducted with protective covers over microplots to exclude rain. Unfortunately, 1985 had a very wet summer and all plots had approximately equal soil water potentials because of lateral movement of water and capillarity. No treatment effects (watering regimes) were observed. However, host resistance effects were observed on infection and inoculum production averaged over water treatments, with greatest infection and inoculum production observed on the susceptible cultivar. Inoculum density was 77, 21, 12, and 8 propagules per gram of soil for the susceptible, low, moderate, and high resistance cultivars, respectively.

Curent research is expanding the effects of different soil types (both suppressive and conducive) on inoculum production by P. p. var. nicotianae. Preliminary results indicate that the suppressive soils (2) greatly inhibit inoculum production and subsequent survival.

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Table 1. Effect of soil type and matric potential on inoculum production of P. p. var. nicotianae

Matric Potential (-bars)	Soil type/Prop. per mg root	
	Grantham	Norfolk
- 0.1	0.9	1.8
- 1.0	5.4	3.2
-10.0	4.5	3.8

Data mean of 4 reps, 4 root systems per rep. Interaction significant at P<0.01.

Table 2. Relationship between symptom development and root zone populations of P. p. var. nicotianae under field conditions.

Cultivar	Date/propagules per gram soil			
	8-9	8-28	9-11	9-17
Hicks	57.4	40.1	30.5	NC
Coker 319	5.4	24.4	42.3	56.4
Coker 411	5.1	10.0	56.4	59.0
Sp. G-28	5.8	9.9	6.2	54.8

Mean of 10 plants per date. Bulked sample containing bulk and rhizosphere soil.

Table 3. Effect of water regime and resistance on inoculum production in microplots.

Cultivar	Water regime/Prop. per gram soil				
	0	1	2	3	x
Hicks	79.2	81.7	75.7	73.2	77.2 a
Coker 319	35.6	9.5	33.7	4.5	20.8 b
Coker 411	18.6	6.0	4.6	16.3	11.6 b
Sp. G-28	12.8	4.1	1.3	2.9	8.3 b
x	36.5	26.8	31.8	24.2	

Mean of eight replications (microplots).

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VII. Papers Presented at Professional Meetings

Shew, H. D. 1985. Inoculum production by Phytophthora parasitica var. nicotianae in roots of flue-cured tobacco. Phytopathology 75:1347.

IX. I acknowledge the technical assistance of D. T. Glover, E. C. Gray, and S. Bhikhai in this study.

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BIOLOGICAL CONTROL OF CERTAIN FOLIAR PLANT DISEASES AND NEMATODES IN TOBACCO

Harvey W. Spurr, Jr.

Professor of Plant Pathology and Lead Scientist,
Tobacco Pest and Biocontrol Research Unit, Agricultural Research Service, USDA in cooperation with N. C. State University
State 05615

I. Summary of Research:

This project is concerned with disease control mechanisms and their application to achieve plant disease control in a practical and economical manner. The emphasis is on new approaches and new techniques for understanding and efficiently controlling diseases. Specific objectives of immediate importance are: determine how to use leaf microflora to control foliar disease, determine how to induce host resistance to disease and develop practical applications, determine how nematodes survive during periods of stress, dormancy and anhydrobiosis.

Research during 1985 was highlighted by the results described in the following paragraphs.

Tobacco blue mold: Sporangiophore and sporangium ontonogeny of Peronospora tabacina was studied using light, scanning, and transmission electron microscopy. The fungus was induced to sporulate by placing 10 mm infected tobacco leaf disks in darkness and 100% humidity. Sporangiophores emerged from stomata within 2 h and continued to elongate for 7 h at which time the first dichotomous branch of sporangiophores was formed. One septum usually developed in the sporangiophores between the first branch and leaf surface and callose-like wall apposition material partially restricted the lumens of the sporangiophores below the epidermal surface at this time. Sporangiophore branching was completed by 8 h and sporangia were initiated synchronously at sporangiophore tips. Septa delimited morphologically immature sporangia from sporangiophore tips at 10 h. All sporangia on an individual sporangiophore were morphologically mature 11 h after the induction of sporulation. Unilaterally umbonate septa containing portions of membranes were observed in intercellular hyphae in close proximity to sporangiophore initials but also occurred randomly throughout the mycelium near the lower leaf surface. (Cooperators: Robert Trigiano, Gerald Van Dyke, C. E. Main)

A new project was initiated entitled: Host specificity and sporulation of Peronospora tabacina, the blue mold pathogen. Specific objectives are: to identify the lectin(s) necessary for the specific interaction of P. tabacina with tobacco leading to colonization of the host, and to determine how humidity induces sporulation on P. tabacina infected leaves. (Cooperator: Mary L. Menetrez)

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Biological control of plant pathogens: Foliar disease biocontrol has been hindered by unpredictable field results. Survival of control agents and their interactions with pathogens occur in a dynamic environment. A systems approach helps to understand these complex processes. One such approach (knowledge-based or "expert" systems) uses a set of decision-making rules (shell) coupled to a knowledge base of facts and observations. A computer simulation of Cercospora leafspot of peanut is the shell component of one such system. Disease progress is predicted as a function of weather, pathogen characteristics, plant growth, and control agents. The knowledge base contains epidemiological parameters, weather data, and results from controlled-environment studies using different biocontrol agent candidates. The model is used to optimize spray timing and dosage, and to predict field results.

Five lyophilized bacterial preparations were evaluated in field trials for survival and efficacy against Cercospora leafspot of peanut (causal agent: Cercospora arachidicola). Bacteria were applied as aqueous suspensions of wettable powders or as dusts, at bi-weekly intervals, to peanut foliage (cv. Florigiant) at three locations in North Carolina and Virginia. Leaflets were periodically sampled to determine survival of applied bacteria and disease progression. Numbers of each bacterial strain declined from 10^{5}-10^{6}/leaflet to 10-10^{3}/leaflet over most 2-wk periods. Background bacterial populations in untreated plots averaged 1.2×10^{4}/leaflet, and consisted mainly of gram-negative rod-shaped organisms. Survival of gram-positive spore-forming bacteria (Bacillus spp.) formulated as wettable powder was less variable than survival of gram-negative, non-sporeforming Pseudomonas cepacia formulated as wettable powder or dust, but P. cepacia controlled disease more effectively. End-of-season disease severity and area under the disease progress curve were significantly lower with several bacterial formulations than in control plots. The bacterial preparations were less effective than the chemical fungicide chlorothalonil. (Cooperators: G. R. Knudsen and Jack Bailey)

III. Postdoctoral Fellows:

Dr. Guy R. Knudsen, ARS-USDA Research Associate, Biological Control of Foliar Pathogens.

Dr. Mary L. Menetrez, Postdoctoral Fellow, Host Specificity and Sporulation of Peronospora tabacina, the Blue Mold Pathogen, Sponsor: R. J. Reynolds Tobacco.

Dr. Wayne M. Thal, Research Affiliate USDA-ARS, Biological Control of Leafspot Diseases, Sponsor: Del Monte Corporation.

IV. Publications:

Knudsen, G. R., Spurr, H. W., Jr. and Bailey, J. E. 1985. A predictive simulation model for peanut leafspot. (Abstr.). Phytopathology 75: 626.

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Spurr, H. W., Jr. and Knudsen, G. R. 1985. Biological control of leaf diseases with bacteria. Pages 45-62 in: C. E. Windels and S. Lindow eds., Biological Control Strategies on the Phylloplane. APS Books, St. Paul, MN.

Spurr, H. W., Jr. 1985. Mode of action of nematicides. Pages 269-276 in: J. N. Sasser ed., An Advanced Treatise on Meloidogyne.

Spurr, H. W., Jr. 1985. Bioassays - critical to biocontrol of plant disease. J. Agric. Entomol. 2:117-122.

Trigiano, R. N., Van Dyke, C. G., Spurr, H. W., Jr. and Main, C. E. 1985. Ultrastructure of sporangiophore and sporangium ontogeny of Peronospora tabacina. Tob. Sci. 29:116-121.

Knudsen, G. R. and Spurr, H. W., Jr. 1985. A computer model for evaluating foliar biocontrol agents. (Abstr.). Phytopathology 75:1343.

Spurr, H. W., Jr. and Knudsen, G. R. 1985. Evaluation of Bacillus thuringiensis for peanut leafspot control in five states. (Abstr.). Proceedings 17th Ann. Meeting Am. Peanut Research Ed. Soc., Inc. p. 46.

Knudsen, G. R. and Spurr, H. W., Jr. 1985. A simulation model explores fungicide application strategies to control peanut leafspot. (Abstr.). Proceedings 17th Ann. Meeting Am. Peanut Research Ed. Soc., Inc. p. 46.

V. Manuscripts Accepted for Publication:

Knudsen, G. R., Spurr, H. W., Jr. and Johnson, C. S. 1986. A computer simulation model for Cercospora Leafspot of Peanut. Phytopathology: (in press)

Knudsen, G. R., Johnson, C. S. and Spurr, H. W., Jr. 1986. A simulation model explores fungicide strategies for control of peanut leafspot. Phytopathology: (in press)

Knudsen, G. R. and Spurr, H. W., Jr. 1986. Management of bacterial populations for foliar disease biocontrol. Indian Press: (in press).

VI. Manuscripts in Review:

Knudsen, G. R. and Spurr, H. W., Jr. 1986. Field survival and efficacy of five bacterial preparations for control of peanut leafspot. Plant Disease: (in press)

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VII. Papers Presented at Professional Meetings:

Knudsen, G. R., Spurr, H. W., Jr. and Bailey, J. E. 1985. A predictive simulation model for peanut leafspot. Potomac Div. Meeting, Am. Phytopathological Soc., Morgantown, W.VA., Mar. 1985.

Knudsen, G. R. and Spurr, H. W., Jr. 1985. A computer model for evaluating foliar biocontrol agents. Ann. Meeting, Am. Phytopathological Soc., Reno, NEV., Aug. 1985.

Spurr, H. W., Jr. and Knudsen, G. R. 1985. Evaluation of Bacillus thuringiensis for peanut leafspot control in five states. Ann. Meeting, Am. Peanut Research Ed. Soc., San Antonio, TX., July 1985.

Knudsen, G. R. and Spurr, H. W., Jr. 1985. A simulation model explores fungicide application strategies to control peanut leafspot. Ann. Meeting, Am. Peanut Research Ed. Soc., San Antonio, TX., July 1985.

IX. Acknowledgements:

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Title: NC 03791 Epidemiology and Control of Foliar Disease of Tobacco

Project Leader: C. E. Main

I. Summary of Research:

This project has the following objectives:

A. Monitor occurrence, distribution, and importance of foliar diseases of tobacco in North Carolina.

B. Determine the source(s) of primary inoculum of Peronospora tabacina.

C. Develop improved control tactics for foliar diseases using fungicides, resistant cultivars, and cultural practices.

D. Monitor the presence of ecotypes, strains and races of foliar pathogens.

E. Relate development and spread of foliar diseases to weather and climate.

F. Assess the impact of foliar diseases on yield and value.

G. Establish cooperative efforts on foliar diseases with state, federal, and international research centers.

Studies on the Blue Mold Disease of Tobacco

Blue mold, caused by the obligate fungus pathogen Peronospora tabacina, continues to be the most serious potential disease threat to the tobacco industry in the United States and Latin America. Macroscale, explosive epidemics generally occur when the growing tobacco crop, the pathogen, and conducive weather conditions co-exist. During 1985-86, scientists at North Carolina State University and the USDA Tobacco Research Laboratory at Oxford, NC studied the biology, epidemiology, development of resistant cultivars, fungicidal control strategy, and fungicide residues using both flue-cured and burley tobaccos.

Objectives:

The objectives of the blue mold project are comprehensive and broad based involving: (1) applied approaches to breeding resistant varieties, formulating fungicidal control strategies and educating the tobacco growers, (2) basic investigations into the genetics and physiology of host resistance, ecologic and genetic variability within the pathogen population, biometeorological aspects of inoculum movement and disease spread, and (3) development of a contingency control program in the event a metalaxyl resistant strain of P. tabacina should be introduced into the United States. This interim progress report describes on-going research on this important disease problem.

Fourteen field experiments were conducted in North Carolina, 9 field tests in Puerto Rico and 7 field tests at three separate locations in Mexico. Blue

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mold occurred in late August in Madison County, NC but did not develop or spread further. Therefore, the North Carolina tests were used mainly to evaluate the type, yield and quality of blue mold resistant breeding lines and to conduct fungicide residue analyses on cured-leaf. Excellent disease evaluation results were obtained in the winter nursery tests at Gurabo, Puerto Rico, and Las Varas, Mexico.

Breeding and Genetics:

The objectives of the host resistance studies in 1985-86 was to: a) evaluate blue mold resistant flue-cured and burley tobacco germplasms with improved agronomic characteristics, and b) identify and characterize additional and/or new sources of blue mold resistance which may be utilized in the development of breeding lines. The long-term goal is to develop tobacco genotypes possessing multiple genetic mechanisms for resistance as a means to increasing the quantitative level of resistance. Such quantitative resistance may be more stable to pathogen variation and less likely to become susceptible to changing populations of P. tabacina.

Several putative blue mold resistant doubled haploid breeding lines (DH's) were selected in 1983-85 at the blue mold breeding nursery located in Gurabo, Puerto Rico. In 1985-86, four flue-cured breeding lines were selected and evaluated for blue mold reaction at Gurabo, Puerto Rico, and at 2 locations in Nayarit, Mexico. Agronomic evaluations were also conducted at the Border Belt Research Station, Whiteville, NC. Three flue-cured lines labelled DH 17, DH 42, and DH 62 were found to be highly tolerant to blue mold and differed significantly in disease reaction from susceptible commercial cultivars. F1 hybrids between DH's and susceptible cultivars had intermediate levels of resistance, but were agronomically superior to the DH lines. Co-dominance of the genes for resistance was evident.

Disease resistance reactions determined in Puerto Rico were corroborated in Mexico. Data obtained in 1985-86 demonstrate that selected DH lines possess genetic resistance under the moderate level of disease pressure experienced at both locations. Evaluations for yield and quality at Whiteville, NC in the absence of blue mold indicated that blue mold tolerant DH's were generally lower yielding than susceptible, commerical cultivars. Nevertheless, the yield of flue-cured breeding line DH 42 did not differ significantly from McNair 944 and possesses acceptable concentrations of nicotine and reducing sugars.

Additional materials including "half-burley" lines (derived from crosses of flue-cured x burley genotypes), burley DH's as well as potential new sources of blue mold resistance, were also evaluated in Puerto Rico and/or Mexico in 1985-86. Three "half-burley" lines - DH 32, DH 35 and DH 90, derived from the cross Ky 17 x Ovens 62, were found to be highly tolerant to blue mold at all locations, but they possess green plant color. Four breeding lines with burley phenotype were also selected in Puerto Rico on the basis of low disease development and will be further evaluated in 1986-87. These are the first burley genotypes to be identified as potentially having blue mold tolerance.

Chemical Mutant, a line developed through chemical mutagenesis in Australia, has been identified as having the highest degree of resistance in the Coresta Test Series. This line was crossed with susceptible cultivars Spt. G-28 and Ky 17, and F1 hybrids and F2 populations were evaluated for blue mold

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reaction in Puerto Rico and Mexico. F1 hybrids appeared more resistant than susceptible parents indicating resistance in Chemical Mutant to be at least partially dominant. Highly resistant genotypes were apparent in segregating F_2 generations. Resistant selections were self-pollinated and crossed to their respective flue-cured or burley parents.

The European lines ZA 684 and ZA 692 were identified as being blue mold tolerant in Puerto Rico and Mexico and will be used in the breeding program as new sources of resistance. Turkish germplasm reported to be blue mold resistant was found to be susceptible in our tests and will be eliminated from the blue mold germplasm pool.

Fungicide Control Strategy:

Tests to evaluate alternative strategies for blue mold control were set up at four locations in North Carolina and at a winter nursery location at Gurabo, Puerto Rico. The disease did not develop at the North Carolina locations, however, some useful information was still obtained. In North Carolina no significant yield effects, either positive or negative, when either metalaxyl as a soil treatment or mancozeb as a routine fungicide spray were used in the absence of the disease. Blue mold did develop in the Puerto Rico tests where two systemic materials, benalaxyl used as a foliar spray, and oxadixyl, used as a preplant plus layby treatment, were each nearly as effective as the standard metalaxyl. Valuable information on the timing of protectant mancozeb applications was also obtained. Good results were obtained where the combined use of host resistance and mancozeb foliar sprays were tested. Corresponding yield and quality data are presently being collected in Puerto Rico for further analysis and additional information. In North Carolina preparations have been made to monitor and test Peronospora tabacina isolates for sensitivity to metalaxyl in the event blue mold develops on metalaxyl treated burley tobacco. Educational information on blue mold was included in various newsletters and during the two Burley Day programs, as well as at other burley field tours and field days.

Cured leaf samples from the burley and flue-cured fungicide tests were saved from all EBDC plots and from individual harvests of selected treatments for residue analysis of EBDC and ETU, a metabolite of EBDC. Chemical analyses of the samples are in progress and should be completed by March 15.

Two approaches to biological control were field tested in Puerto Rico during 1985-86. These included induced host resistance resulting from sodium selenite sprays or TMV inoculation of a hypersensitive cultivar and spray application of two bacterial antagonists. All treatments provided a significant measure of control at the first evaluation after the appearance of blue mold. At the second evaluation it was evident these treatments were not withstanding the pressure of the epidemic. Analysis of these results in relation to rate of epidemic development indicates a need to shorten the timing and increase the frequency of the biological treatments in relation to disease progress. Research to improve antagonist survival and efficacy by formulation and selection is progressing.

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Epidemiology and Biometeorology:

Remodeling and improvements in the NCSU Phytotron "confinement facility" were completed. Controlled environment studies on "systemic" blue mold indicates that this important phase of the disease is a function of plant age, inoculum pressure, site of plant infection and environment rather than the result of some unique biotype of the pathogen. Two trips were made to the USDA Ft. Dietrick Plant Pathology Laboratories in Frederick, MD to conduct genetic tests on the asexual spores of the P. tabacina using a biochemical (isoenzyme) analysis. The results to date show little or no genetic diversity exists in the US population. Fourteen pathogen isolates, collected from widely scattered U.S. locations over six years, were nearly identical for loci coding for approximately 32 enzymes generally considered characteristic of positive genetic diversity. Also, lack of heterogeneity in the population is taken as evidence that the sexual oospore is not actively functioning in the United States, thereby not contributing to genetic or ecological variability. At present, nothing is known about the status of the oospore and recombination of fungus genes in Mexico or the Caribbean. A recent trip to Mexico by NCSU and USDA scientists provided an opportunity to sample blue mold infected tobacco leaves in both the east and west coast production areas. These samples are being processed for histological examination. The objective is to record the frequency, size and morphology of oospores in necrotic tissue. This information is vital to understanding the role of the sexual cycle to survival and variability of P. tabacina. These results are preliminary and an expanded and more comprehensive study is warranted.

A biochemical/physiological investigation has been initiated at the USDA Tobacco Laboratory, Oxford, NC on the interaction between the tobacco leaf surface and the sporangiospores of P. tabacina. This specificity/recognition phenomenon, involving tobacco lectins and a surface carbohydrate, should shed light on the infection process determining disease development and the nature of host resistance. A second phase of this post-doctoral research will investigate in detail the critical role atmospheric humidity plays in triggering sporulation by the fungus pathogen following infection.

Biometeorological studies have focused on long-distance, airborne movement of inoculum (asexual fungus spores) necessary for new disease outbreaks each year and continued spread of the epidemic. A computer-assisted mapping technique, i.e. meteorological trajectory analysis, is being developed at NCSU that permits the tracking and documentation of probable inoculum pathways and identification of source regions. The SE United States, SW Texas and Mexico are all known or suspected sources of inoculum for North Carolina.

Host-pathogen-environment simulation models have proven to be a unique experimental approach to evaluating existing knowledge on plant diseases, identifying data gaps and planning for future research activities on complicated biological systems. Over the past four years sufficient data has been accumulated to initiate the construction of a "Blue Mold Simulation Model" based upon the biology of the disease. The goal would be to develop the model using information on forecasting, chemical control strategies and formal decision analyses to generate control recommendations for flue-cured and burley tobaccos. As we learn more about the genetic variability of the pathogen and the nature of host resistance, the model could also be used to better understand the dynamics of blue mold epidemics per se. It is anticipated that as the model construction

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proceeds, additional information and relationships would need to be investigated using Phytotron and field studies. The benefits accruing from such an approach would be considerable and the model could serve as a focus for establishing priorities for future research.

The Texas/Mexico Situation--An Inoculum Source?

The blue mold situation that has developed in Mexico and certain Latin American countries warrants our serious attention. One or more metalaxyl resistant strain(s) of P. tabacina have been documented to occur on cultivated tobacco in the Gulf coast production areas of Mexico. Since 1980 the systemic fungicide metalaxyl has provided excellent control of blue mold in North Carolina. In the eventuality that these new fungus strains be air-transported into the U.S., via a spatial bridge of wild Nicotianae repanda in SW Texas or by some other route, an emergency situation could exist. Presently, 75-80 percent of our growers rely almost entirely on metalaxyl for blue mold control. Contingency plans involving protectant fungicides have been developed by our NCSU scientists should this happen. During the fall-winter season, selected NCSU blue mold resistant breeding lines and an international set of differential tobacco cultivars (CORESTA collection) were evaluated at three separate locations in Mexico under disease pressure of the resistant pathogen strain. From February 16-22 three scientists from NCSU and two research managers from R. J. Reynolds Company visited each of the locations to evaluate the tests and to observe first hand the situation related to metalaxyl resistant blue mold. These were preliminary tests and although the results were not conclusive, the opportunity to continue the investigation, generate valuable information and gain lead time in preparing our growers was apparent. Mexico has a serious blue mold problem. The sooner they solve their problem the less risk there will be to our North Carolina tobacco industry.

Acknowledgements

The blue mold epidemic is a complex biological phenomenon that can result in serious economic impact on North Carolina growers and the tobacco industry. History has proven this to be the case. The blue mold program at North Carolina State University is committed to finding practical solutions that should lessen the risk our farmers face each year. At the same time, very basic research on the nature of the host-pathogen-environment components of the epidemic are being investigated. The blue mold-tobacco system represents one of the most promising case studies for phytopathological research available today. The challenges are many and the opportunities are exciting. The expertise and efforts of some ten scientists are involved in the research described in this report. They should be recognized as:

J. M. Davis	Meteorology and Plant Pathology
Mary Menetrez	Post-doctorate, USDA, Oxford
M. A. Moss	Ph.D. student, Plant Pathology
N. T. Powell	Extension/Research, Plant Pathology, Flue-cured
Rebeca Rufty	Crop Science
T. J. Sheets	Pesticide Residue Research Laboratory
P. B. Shoemaker	Extension/Research, Plant Pathology, Burley
H. W. Spurr, Jr.	USDA Oxford, Plant Pathology
E. A. Wernsman	Crop Science
C. E. Main	Coordinator, Plant Pathology

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The interest and support of the R. J. Reynolds company is gratefully acknowledged, particularly the input of Richard Reich, Arnold Mitchem and Jerry Shiffert.

II. Graduate Students: Michael A. Moss

Epidemiology of the blue mold diseases of tobacco caused by Peronospora tabacina

III. Postdoctoral Fellows: None

IV. Publications:

Main, C. E., J. M. Davis, and M. A. Moss. 1986. The biometeorology of blue mold of tobacco: A case study in the epidemiology of the disease. pp. 453-472. IN: D. R. MacKenzie, C. Barfield, T. G. Kennedy, and R. D. Berger (eds.). Movement and Dispersal of Agriculturally Important Biotic Agents. Claitor's Publ. Co., Baton Rouge, LA. 611 p.

Davis, J. M., C. E. Main, and W. C. Nesmith. 1986. The biometerology of blue mold of tobacco. The evidence for long-range sporangiospore transport. pp.473-498 IN: D. R. MacKenzie, C. Barfield, T. G. Kennedy, and R. D. Berger (eds.). Movement and Dispersal of Agriculturally Important Biotic Agents. Claitor's Publ. Co., Baton Rouge, LA. 611 p.

Shew, H. D., and C. E. Main. 1985. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina. Plant Disease:69:901-903.

V. Manuscripts Accepted for Publication:

Davis, J. M., and C. E. Main. Applying atmospheric trajectory analysis to problems in epidemiology. (Feature article.) Plant Disease (to appear in spring, 1986).

VI. Manuscripts in Review:

Moss, M. A., C. E. Main, and J. M. Davis. Evidence of temperature tolerance in Peronospora tabacina. Phytopathology.

Moss, M. A., and C. E. Main. The effect of temperature on sporulation and viability of Peronospora tabacina isolates collected in the United States. Phytopathology.

Moss, M. A., and C. E. Main. Factors affecting systemic infection of tobacco by Peronospora tabacina. Phytopathology.

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Kirby, H. W., and C. E. Main. Identification and analysis of factors related to acceptance of integrated pest management among tobacco farmers. Plant Disease.

VII. Papers presented at Professional Meetings:

Moss, M. A., and C. E. Main. 1985. Temperature tolerance of Peronospora tabacina in the U.S. (Abstr.) APS Annual Meeting, Reno, NV. August 11-15, 1985.

Davis, J. M., C. E. Main, and W. C. Nesmith. 1985. Evidence for long-range inoculum transport of Peronospora tabacina. (Abstr.) APS Annual Meeting, Reno, NV. August 11-15, 1985.

Shew, H. D., and C. E. Main. 1985. Leaf spot of tobacco caused by Rhizoctonia solani. (Abstr.) APS Annual Meeting, Reno, NV. August 11-15, 1985.

Kirby, H. W., and C. E. Main. 1985. Assessment of farmer utilization of tobacco integrated pest management programs. APS Annual Meeting, Reno, NV. August 11-15, 1985.

VIII. Graduate Student Theses Completed During Reporting Period:

Michael A. Moss. Epidemiology of tobacco blue mold. Doctor of Philosophy Thesis, North Carolina State University, Raleigh, NC.

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Title: NC05507 Disease Control Strategies for Burley Tobacco and Vegetables in Western North Carolina

Project Leader: P. B. Shoemaker

I. Summary of Research:

Alternative fungicides and strategies for blue mold control (with C. E. Main). Tests to evaluate alternative strategies for blue mold control were set up at three locations in North Carolina and at a fourth location at Gurabo, Puerto Rico. The disease did not develop at the North Carolina locations, however, some useful information was still obtained. No beneficial or detrimental yield effects were observed by use of either metalaxyl (Ridomil®) as a soil treatment or mancozeb as a routine fungicide spray in the absence of the disease. Blue mold did develop in the Gurabo tests where two systemic materials, benalaxyl used as a foliar spray, and oxadixyl, used as a preplant plus layby treatment, were each nearly as effective as the standard metalaxyl. Additional information on the timing of protectant mancozeb applications was also obtained. Preparations were made to monitor and test Peronospora tabacina isolates for sensitivity to metalaxyl in the event blue mold developed on metalaxyl treated burley. Blue mold was found on untreated tobacco in one small area of one county in late August. Metalaxyl resistance was not suspected so tests for resistance were not made. Educational information on blue mold were included in various newsletters and during the Burley Day program, and other burley field tours and field days.

Burley soil-borne disease survey (with H. D. Shew). Fields with a history of poor growth or stunting were surveyed in the burley area to determine quantitative levels of certain soil-borne pathogens, including: Thielaviopsis basicola (black root rot), Phytophthora parasitica (black shank) and Meloidogyne spp (root knot). Thirteen survey farms were selected by the respective county agent in five counties, including Haywood, Buncombe, Madison, Yancey and Mitchell. Twenty fields were divided into 1 to 4 sampling areas each depending on size and variation in terrain. Soil samples were taken in early May and then assayed for the different organisms soon thereafter in Raleigh. In addition, Judy's Pride plants were set in each sampling area (up to 16 per field) in late May and were pulled and inspected for black root rot symptoms in late June. Growing conditions were unseasonably dry and warm from late May through June. Even with relatively unfavorable conditions for disease development, black root rot readily developed on the susceptible Judy's Pride indicator plants, which showed severe stunting, wilting and blackened lesions and root rotting on affected roots. Soil assay levels ranged from 0 to as high as 352 T. basicola propagules per gram of soil. Where soil assay results were 0 or very low, the Judy's Pride plants were normal and did not have black root rot symptoms. Where soil assays resulted in high counts, Judy's Pride plants were stunted and showed root rot symptoms in relation to the recovery of the organism. In addition, where the counts were high, some wilting and root rot symptoms were observed on the grower's cultivar, usually Ky 14, which possesses high black root rot resistance. Soil assays for the black shank organism (Phytophthora parasitica) and for root knot nematodes (Meloidogyne spp) were negative in all the fields in the survey. Soil pH ranged from a low of 5.4 to a high of 6.5. Soil

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assays for T. basicola for three pH regimes: low (pH 5.4-5.7), medium (pH 5.8-6.1) and high (pH 6.2-6.5) averaged respectively, 40, 84 and 96 propagules per gram of soil. This survey establishes quantitative levels for the black root rot organism, Thielaviopsis basicola, in selected fields with a history of stunting and poor growth. A more comprehensive survey and field tests to determine yield losses due to this disease are planned for the future.

IV. Publications

Shoemaker, P. B. 1985. Foliar applied systemic fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 40:164.

Shoemaker, P. B. 1985. Soil applied systemic fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 40:165.

Shoemaker, P. B. 1985. Protectant fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 40:165.

VII. Papers Presented at Professional Meetings:

Shelton, J. E. and Shoemaker, P. B. 1985. Effects of soil fumigation on urea as a nitrogen source for burley tobacco. Tobacco Workers Conf. Jan 7-10.

Shoemaker, P. B. and Main, C. E. 1985. Fungicides and strategies for blue mold control. Tobacco Workers Conf., Jan 7-10.

IX. Acknowledgements: I wish to thank: Superintendents J. E. Edwards, D. F. Tugman, respectively, Mountain Research Station, Waynesville and Upper Mountain Research Station, Laurel Springs and their staffs for on-site assistance, especially Hugh Russell; various companies, including, Ciba-Geigy, Montedison USA, Rohm & Haas, SDS Biotech, and R. J. Reynolds for materials and financial assistance; and especially M. H. Henry and D. C. Milks for their technical assistance and expertise.

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NC 3901 PLANT BED AND FIELD MANAGEMENT

OF FLUE-CURED TOBACCO

G.S. Miner, Project Leader
J.W. Wade, Research Technician

I. Summary of Research:

A. Prevention of TMV transmission in plant beds: Clipping flue-cured tobacco transplants to improve transplant uniformity is becoming widespread in North Carolina but introduction of tobacco moaic virus (TMV) by contact remains a potential threat to adoption of this practice. A study was undertaken in cooperation with Dr. Guy Gooding to investigate means of preventing transmission of TMV to plantbeds by contact.

Washing a TMV-contaminated forefinger with any of seven commercial handsoaps reduced lesion development on Burly 21 from 176 to about 1 lesion/ $25cm^2$ leaf surface (Table 1). While lava soap has been reported to be an effective agent for decontaminating hands, other soaps were as effective in this study. While TMV transmission by hand can be minimized, there appears to be no means of completely eliminating transmission by contaminated hands.

Clipping plots (2.5 yd^2) with a TMV-contaminated mower resulted in transmission of TMV to about 35% of the plants (Table 2). Decontamination of the infected mower with 2.5% NaClO completely eliminated transfer of TMV to plants by clipping but 0.5% NaClO did not. Supporting work has shown that a NaClO concentration of at least 2.5% is required to solubilize dried tobacco juice on metal. Recommendations for mower decontamination should include the use of NaClO with at least a 2.5% concentration.

Attempts were made to prevent the spread of TMV by clipping when it is present in the plantbed before clipping. However, all preventative treatments were unsuccessful (Tables 3 & 4). Thus, hand and mower sanitation procedures must be implemented to prevent the introduction and spread of TMV in plantbeds. The presence of TMV in the plantbed at the time of clipping remains an obstacle to the use of clipping as a management practice.

B. Effect of rate of fertilizer application on salt levels in the soil and on transplant production under plastic plantbed covers.

Planted experiments were established at three locations in 1985 to evaluate the effects of rate of fertilizer on soil salt levels and tobacco transplant development under plastic covers. Treatments consisted of 20, 35, 50, 65, 80, and 95 lb/100 yd^2 of 12-6-6 fertilizer applied in the fall with four replications.

Salt levels of the saturated soil extract at Whiteville averaged 0.8 mmhos/cm^2 across treatments. This low salt level and the absence of treatment differences was due to removal of the cover 3-4 weeks before

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seeding and leaching of salt by subsequent rainfall. Mean seedling production was 92% of seeds planted. At Clayton, mean salt levels at seeding was 23.5 mmhos/cm^2 and ranged from 16.7 to 32.2 mmhos/cm^2 for the 20 and 95 lb/100 yd^2 rates of fertilizer, respectively. The irrigation after seeding reduced mean salt levels one week later to 3 mmho/cm^2 with no differences among treatments. Mean plant production was 75% of the seeds planted. At Oxford, mean salt levels were 51 mmhos/cm^2 at seeding and were reduced by irrigation to only 34 mmho/cm^2 one week later. The amount of water was insufficient resulting in a mean plant production of only 44% of seeds planted. Thus, it is obvious that the extent of fertilizer salt injury to seedlings in plantbeds is a function of the amount of irrigation water applied and it emphasizes the importance of irrigating plantbeds at seeding to remove fertilizer salts.

VII.

II. Graduate Students:

One M.S. Candidate
One M.S. Candidate co-chaired with Dr. Raper
One Ph.D. Candidate co-chaired with Dr. Peedin

III. Post Doctoral Fellows:

None

IV. Publications:

Vepraskas, M.J. and G.S. Miner 1986. Effects of subsoiling and mechanical impedance on tobacco root growth. Soil Sci. Soc. Am. J. Vol. 50 (In Press).

Miner, G.S., G.V. Gooding, Jr., and J.M. Moore 1986. Prevention of mechanical transmission of tobacco mosaic virus in tobacco plantbeds. Tob. Sci. March (In Press).

V. Manuscripts in Review:

Vepraskas, M.J., G.S. Miner, and G.F. Peedin. Relationships among relative tobacco yield increase to subsoiling and soil physical properties. Agron. J.

Vepraskas, M.J., G.S. Miner, and G.F. Peedin. Relationships of tillage pans, soil properties, and subsoiling to root growth. Soil Sci. Soc. Am. J.

Smith, W.D., G.F. Peedin, W.K. Collins, M.R. Tucker, G.S. Miner, and E.J. Kamprath. Tobacco response to sulfur on soils differing in depth to the angillic horizon. Agron. J.

VI. Papers Presented at Professional Meetings:

Vepraskas, M.J., P.H. Hardy, and G.S. Miner. Effect of soil physical and chemical properties on tobacco yield response to subsoiling.

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Presented at 31st Tobacco Workers Conference, Jan. 7-10, 1985 at Pinehurst, NC.

Vepraskas, M.J., G.S. Miner, and G.F. Peedin. Relationship of soil physical properties to tobacco yield response to subsoiling. Presented at Am. Soc. Agron. meetings, Dec. 2-6, 1985 at Chicago, IL.

VII. Graduate Student Theses Completed During the Reporting Period:

Hardy, D.H. An investigation of subsoil fertility and its effects on yield and nutrient content of flue-cured tobacco.

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Table 1. Effectiveness of hand-decontamination treatments on the development of local lesions of tobacco mosaic virus infection on Burley 21 tobacco.

Treatment	Lesion Development[a] (Lesions/25cm^2 of leaf surface)
Crude juice	176.5 a[b]
Water	17.0 b
Safeguard	1.3 c
Ivory	1.2 c
Palmolive	1.2 c
Lava	0.8 c
Coast	0.7 c
Dial	0.7 c
Go-Jo	0.7 c
Check	0.0 c

[a]Values represent mean numbers of local lesions per 125 cm^2 leaf surface on six leaves.
[b]Means in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

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Table 2. Effect of various mower decontamination treatments on the incidence of infected plants in plantbeds after clipping 1985.[a]

Treatment Code[b]	TMV-Infected Plants (%)[c]
Check	0.0 b
TMV-T-clip	34.7 a
TMV-T-0.5% sodium hypochlorite	3.7 b
TMV-T-2.5% sodium hypochlorite	0.0 b
Fall TMV-HN-clip	0.5 b

[a]Beds were sprayed with water to the point of runoff immediately before clipping treatments to simulate early-morning effects of dew accumulation.

[b]Check = not clipped; TMV-T = mower used to clip TMV-infected tobacco; 0.5 and 2.5% sodium hypochlorite = mower sprayed with respective solution; Fall-TMV-HN = mower used to clip TMV-infected horsenettle in fall of previous year and stored until clipping time (all other treatments imposed at clipping time).

[c]Means (3 replications) in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

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Table 3. Effect of clipping with and without the use of milk and papain on the spread of tobacco mosaic virus in tobacco plantbeds at 3 locations in 1982.

Preventative Treatment	Clip	TMV-Infected Plants(%)[a]			Mean
		BBTRS[b]	OTRS	CCRS	
None	No	0	0	0	0
None	Yes	63	83	71	72
Milk(2%)	No	0	0	0	0
Milk(2%)	Yes	54	54	68	59
Papain(1%)	No	0	0	0	0
Papain(1%)	Yes	40	50	47	46

[a]Inoculum consisted of four seedlings/plot that were inoculated with TMV two weeks prior to clipping. These plants were excluded from the data.

[b]BBTRS=Border Belt Tobacco Research Station; OTRS=Oxford Tobacco Research Station; CCRS=Central Crops Research Station.

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Table 4. Effect of clipping and various preventative measures on the spread of tobacco mosaic virus in tobacco plantbeds in 1985.

Preventative Applied	TMV-Infected Plants(%)[a]			
	Check (not clipped)	Clip	Spray Through Deck	Spray Before Clipping
None	0	93	-	-
Water	-	-	97	96
Milk (10%)	-	-	-	92
NaClO (0.25%)	-	-	99	97
NaClO (0.5%)	-	-	91	95
NaClO (0.75%)	-	-	98	93
NaClO (1.0%)	-	-	89	91
Poke weed juice (10%)	-	-	77	48
Poke weed Juice (20%)	-	-	66	53

[a]Inoculum consisted of four seedlings/plot that were inoculated with TMV two weeks prior to clipping. These plants were excluded from the data.

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NC 03659 ENVIRONMENTAL DETERMINANTS OF

CROP GROWTH AND SOIL PRODUCTIVITY

C.D. Raper, Jr., Project Leader

I. Summary of Research:

A. Regulation of Nitrogen Uptake at the Whole-Plant Level.

In previous work with intact soybean plants grown in flowing hydroponic culture under phytotron conditions, uptake rates of nitrogen from solution containing 1.0mM nitrate was shown to vary during a 4-week period in a cyclic pattern with a frequency of 3 to 4 days between maximum rates of uptake. In a continuation of the study, nitrogen uptake was monitored for soybean and tobacco plants growing in solutions containing 1.0 and 10.0 mM nitrate. The cyclic pattern of variations in uptake rates was observed at both nitrate concentrations. Preliminary results indicate similar patterns of uptake from solutions containing 1.0 and 10.0 mM ammonium. Although maximum rates of nitrogen uptake from the solutions were substantially greater at the 10.0mM than the 1.0 mM concentration of nitrate, a net efflux of nitrate occurred during the uptake minima. Since the amplitudes of both the uptake maxima and minima were increased during each oscillation and the frequency of oscillation remained the same, the total accumulation of nitrogen in the plants was relatively unaffected by solution concentration. When root-zone temperature was controlled at 14 and 24^{0} C, the periodicity of oscillations was relatively unaffected although amplitudes of the maxima were reduced at the lower temperature. Collectively, these data indicate that a major component of regulation of nitrogen uptake by roots operates at the shoot level and possibly is associated with physiological and morphological demand for nitrogen by the shoot. To test this hypothesis, experiments have been conducted in which shoot activity was altered by decreasing day/night temperatures from 26/22^{0}C to 18/14^{0}C. Rates of leaf emergence and enlargement were reduced at the cooler aerial temperature, while net photosynthetic rate per unit leaf area was only slightly affected. If the periodicity of nitrogen uptake rates is associated with rate of leaf emergence, as we postulate, then the periodicity of oscillations in uptake rate by roots should be lengthened at the 18/14^{0}C aerial temperature.

B. Effect of Nitrogen Stress on Partitioning of Nitrogen and Dry Weight

In previous studies, we have shown that when nitrogen is resupplied, photosynthetic rates, as well as rates of leaf emergence and expansion of nitrogen-stressed leaves of soybean and tobacco return within a few days to values comparable to those for leaves of nonstressed plants when plants are growing at a photosynthetic photon flux density (PPFD) of 700 umol $m^{-2}s^{-1}$. In a subsequent study, plants were grown at a PPFD of 350 umol $m^{-2}s^{-1}$ to determine how a change in photosynthesis alters partitioning of dry matter and response to nitrogen stress. For nonstressed plants at the lower PPFD, photosynthetic rate per unit leaf area was 30% lower, and leaf area was about 50% lower, than for the nonstressed plants at the higher PPFD. For

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nitrogen-stressed plants under the lower PPFD, when nitrogen was resupplied, photosynthetic rates per unit leaf area returned to the rate for nonstressed plants, but the recovery of rates of leaf emergence and expansion at the lower PPFD was delayed relative to the time for recovery at the higher PPFD.

C. Utilization of Ammonium as a Nitrogen Source.

Utilization of ammonium is enhanced when acidification of the root-zone, which results from an excess influx of cations relative to anions during absorption of ammonium, is alleviated. Both soybean and tomato plants can utilize ammonium as effectively as nitrate when acidity of the solution is controlled at pH 6.0. When acidity of the solution is reduced, however, uptake rates of ammonium per unit root mass are reduced. To test the hypothesis that the reductions in growth often reported for ammonium-fed plants are a consequence of nitrogen stress within the plant resulting from the reduction in uptake rates as acidity increases during uptake, soybean and tobacco plants were grown for 3 weeks in hydroponic culture containing 1.0mM ammonium as the sole source of nitrogen. Acidities of the culture solutions were controlled at pH 6.1, 5.1, and 4.1 by automatic additions of a dilute base of acid. When acidity was increased from pH 6.1 to 5.1, accumulation of dry matter and nitrogen were reduced by about 40% within 2 weeks. Net photosynthetic rates per unit leaf area, however, were not affected, and the decreased growth was associated with reductions in rates of appearance and expansion of new leaves. Uptake rates of ammonium per unit root mass were consistently about 25% lower throughout the 3 weeks at pH 5.1 than at 6.1. A further increase in solution acidity from pH 5.1 to 4.1 resulted in cessation of net dry matter production and appearance of new leaves within 10 days. Net photosynthetic rates per unit leaf area declined rapidly until all viable leaves had abscissed by 18 days. Uptake rates of ammonium, which initially were about 50% lower at pH 4.1 than at 6.1, continued to decline with time of exposure until net uptake ceased at 2 weeks. These responses, which are characteristic of the sequence of responses that are observed during onset and progression of a nitrogen stress, lend support to the hypothesis that reductions in growth associated with ammonium nutrition begin with the reduction in uptake rate of ammonium per unit root mass in response to acidification of the root-zone.

II. Publications:

Peet, M.M., C.D. Raper, Jr., L.C. Tolley, and W.P. Robarge 1985. Tomato responses to ammonium and nitrate nutrition under controlled root-zone pH. J. Plant Nut. 8:787-798.

Tolley, L.C., and C.D. Raper, Jr. 1985. Cyclic variations in nitrogen uptake rate in soybean plants. Plant Physiol. 78:320-322.

III. Graduate Students:

Tom C. Granato (Ph.D.)

IV. Postdoctoral Fellows:

Dr. Leslie Tolley-Henry

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Title: NCO 3827 Cultural, Nutritional and Varietal Investigation with Burley Tobacco

Project Leader: J. E. Shelton

I. Summary of Research

This project has the following objectives:

A. To evaluate methods of distinguishing differences in P and K requirements of soils with differing chemical and physical characteristics.

B. To correlate information from objective A with yield and quality of burley over a wide range of soil P and K levels and soil management systems.

C. To investigate the use of urea as a N source for burley under different soil management systems.

Rates of 0, 85, 170 or 225 kg ha^{-1} P_2O_5 applied in 1984 resulted in soil test levels of 134, 152, 182 and 223 mg P/dm^3. The efficiency of the three rates of P application in increasing soil test P was 0.21, 0.28 and 0.35 mg P/dm^3 for each unit of P applied. Maximum yields in 1984 were obtained at the highest P levels. In 1985 these plots were split with either 0 or 57 kg ha^{-1} P_2O_5 being applied. There were no significant differences in yield from the 57 kg ha^{-1} P_2O_5 except at the initial 0 rate of application. In a greenhouse study using soil obtained from these plots, maximum yields were obtained at the 182 mg P/dm^3 soil test level. Two additional sites representing different soil types were established in 1985. Yield results from these tests were variable by location and soil test level. Detailed laboratory and greenhouse studies are incomplete.

Potash rates of 0, 114, 228 or 456 kg ha^{-1} on a low K soil resulted in yields of 2838, 3111, 3221 and 3258 kg ha^{-1} respectively. On a soil high in soil test K rates of K_2O equivalent to 0, 85 and 170 kg ha^{-1} resulted in non-significant yields of 3257, 3519 and 3363 kg ha^{-1}. The recommended rate of K_2O ha^{-1} would have been 262 and 95 kg ha^{-1} for these soil conditions. Potash application rates, required to achieve maximum yields, based upon a 5% K saturation of the exchange capacity would have been 228 and 0 kg ha^{-1} K_2O for these two soil situations. Detailed greenhouse studies on these and other soils using the percent saturation concept will be conducted.

Nitrogen source-rate studies were conducted at 2 locations in Alleghany County using Urea, NH_4NO_3, and $NaNO_3$ at rates of 170, 225 and 280 kg N ha^{-1}. The 280 kg ha^{-1} applied as $NaNO_3$ resulted in high soluble salts and some early plant stunting due to extremely dry weather. However, plants recovered and there was no significant difference in yield or quality from nitrogen sources. Although there was some variation between N sources, there was a significant increase in yield between 170 and 225 kg N ha^{-1} with no difference between 225 and 280 kg N ha^{-1}.

Nitrogen rates of 170, 225, 280 and 340 kg N ha^{-1} applied to a sandy loam soil was disced in and raised beds prepared using a disc bedder.

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Irrigation was used to supply a minimum of 3.5 cm of water per week during the tobacco growth period. Under those conditions of soil type and management system, significant yield increases were obtained up to the 380 kg N ha^{-1} rate of application.

VII. Papers presented at Professional Meetings:

The 31st Tobacco Workers Conference. Jan 7-10, Pinehurst, NC

Shelton, J. E. Effect of Bedding on Response of Burley Tobacco to Nitrogen Fertilization.

Shelton, J. E. and P. B. Shoemaker. Effect of Soil Fumigants on Urea as a Nitrogen Source for Burley Tobacco.

IX. Acknowledgements:

Appreciation is expressed to Hugh Russell and Dwayne Tate for their valuable assistance in conducting these studies. Also appreciation is expressed to the burley extension agents and grower cooperators.

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Title: NC 3819 COVER CROP EVALUATION AND NO-TILL CROP PRODUCTION ON MOUNTAIN SOILS OF NORTH CAROLINA.

Project Leader: G.D. Hoyt

1. Summary of Research:

This project has the following objectives:

A. To establish conservation tillage for burley tobacco production in the mountain region on soils subject to erosion.

B. To determine optimum fertilizer source and placement for conservation tilled burley tobacco.

C. To determine the effect of various tillage systems on tobacco growth, yield, and root development.

D. To determine optimum spring cover crop plowing date for maximum fertilizer efficiency and tobacco yields.

Various management systems were established in 1985 to further evaluate the consequences of conservation tillage and cover crop residues on tobacco production. Previous research concluded that rye or barley residue utilized with a conservation tillage program could provide competative yields with conventional culture and provide needed reduction in erosion. Although yields are comparable at the end of a growing season, early transplant vigor is reduced when conservation tillage is applied. Current research now addresses this subject (vigor reduction) by various fertilizer schemes for supplying adequate nutrition early in the season. The first experiment was established using various N sources and/or in combination with phosphorus in strip-tilled tobacco. Yield results were not significantly different nor was tobacco quality affected by the various fertilizers sources (Table 1).

In the second experiment three tillage systems were compared using conventional tillage, strip-tillage, and no-tillage as treatments. Although late season recovery of crop growth parameters has been common in conservation tillage (as in this year's tobacco yields, Table 2), early season measurements have continuously shown vigor reduction. Both tobacco height early in the season (not shown) and flowering date (Table 2) indicates the rather poor performance of the tobacco crop both in strip-till and no-till culture. High transplant survival in the strip-tilled treatment does indicate a good growing environment (water) for plants in that culture.

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The third tobacco experiment was designed to supply information on the effect of incorporated cover crop residue on conventional tilled tobacco yields. Early plowing in the mountains has continually produced excessive erosion of topsoil during early spring months. Yield results for the four plowing dates indicates higher yields by plowing in December, then equivalent yields during Febuary through April (Table 3). Lower tobacco heights measured early in the growing season (April treatment) indicates a possible nutrient tie-up with the residues that were plowed under.

V. Manuscripts accepted

Hoyt, G.D. and W.L. Hargrove. 1986. Legume cover crops for improved crop and soil management in the southern U.S. HortScience: In Press.

VII. Papers presented at professional meetings:

A. Winter cover crops for no-till tobacco production. Presented before the Burley Section, 31st Tobacco Workers Conference at Pinehurst, N.C., January, 1985.

B. Cover crop residues for strip-tilled tobacco and vegetable production. G.D. Hoyt, T.R. Konsler, R.G. Gardner, J.E. Shelton and F.B. Haynes. Presented as a poster paper before the 1985 Southern No-till Conf. Experiment, Ga., July, 1985.

C. Conservation tillage for vegetable and tobacco production. G.D. Hoyt. Presented before the International Soil Tillage Research Organization 10th Conf. Guelph, Ontario, July, 1985.

XI. Acknowledgements:

I wish to thank Mr. Anthony Cole for his excellent technical assistance on this project.

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TABLE 1. The effect of fertilizer source on strip-till tobacco yield and grade index.

Fertilizer	%NH_4	%NO_3	Yield lbs/A	Grade Index
46-0-0	100	0	2300	.96
30% N Soln	75	25	2350	.96
33.5-0-0	50	50	2430	.96
16.5-0-0	0	100	2270	.96
10-34-0			2400	.97
18-46-0			2380	.96
LSD (.05)			NS	NS

TABLE 2. The effect of tillage on tobacco growth, yield and grade index.

Tillage	Transplant survival	% Flower day 98	Yield lbs/A	Grade index
Conventional	85.0	76	2425	.93
Strip-till	87.8	30	2225	.93
No-till	79.6	27	2244	.93
LSD (.05)	NS	31	NS	NS

Table 3. The effect of plowing date on tobacco growth, yield and grade index.

Plowing Date	Tobacco height (cm) 45 days	75 days	Yield	Grade index
December	43.4	134.8	3460	.96
February	40.8	135.2	3170	.96
March	43.2	140.1	3030	.97
April	27.9	105.5	3190	.97
LSD(#)	13.1 **	25.7 **	259 *	NS

# indicates level of significance: * = (.10), ** = (.01)

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TOBACCO LITERATURE SERVICE

Annual Report for the Calendar Year 1985

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		1984	1985	Change
I.	TOBACCO ABSTRACTS.			
	Abstracts published	1,357	1,285	-72
	Publications searched	5,580	5,964	+384
	Average per month	465	497	+32
	Distribution			
	Domestic:			
	Exchange	48	49	+1
	Complimentary	53	109	+56
	Subscriptions	106	115	+9
	Total	207	273	+66
	Foreign:			
	Exchange	140	144	+4
	Complimentary	27	27	Nc
	Subscriptions	151	152	+1
	Total	318	323	+5
	Total, All Categories	525	596	+71
	Copies Per Year	3,150	2,980	-170
II.	TOBACCO REPRINT SERIES.			
	Reprints published (#707-721)	17	14	-3
	Recipients	164	160	-4
	Copies distributed	2,975	2,240	-735
III.	BIBLIOGRAPHIC INFORMATION SERVICE			
	Request served	1,050	1,100	+50
IV.	PHOTOCOPY SERVICE.			
	Tobacco Staff	6,074p.	6,950p.	+876
	Paid Orders	1,067p.	525p.	-542

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V. OTHER PUBLICATIONS DISTRIBUTED.

"Recent Advances in Tobacco Science".	59	69	+10
"Theses on Tobacco. I. North Carolina State University. II. United States Universities" by Carmen M. Marin.	80	4	-76
"Tobacco Literature, a Bibliography" by Carmen M. Marin	35	4	-31

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ADDENDUM

Listed below, by department, are research activities which received supplemental funding from the North Carolina Tobacco Foundation, Inc. for the period July 1, 1985 through June 30, 1986.

Activity	Investigator(s)
Biochemistry	
Development of Ripening Agents for Tobacco	E. C. Sisler
Biological and Agricultural Engineering	
Development of Fourier Transform Instrumentation for Rapid Chemical Analyses of Tobacco	W. F. McClure W. W. Weeks F. G. Giesbrecht
Stress Physiology of Tobacco Seedling Development	S. C. Mohapatra
Proper Curing Humidities and Temperature Elevation Rates for Top Quality and Minimum Fuel Cost	R. W. Watkins
Burley Mechanization	C. W. Suggs
Botany	
Cytohistological and Anatomical Changes in the Apical Meristem of Tobacco During the Transition to Flowering	Judith F. Thomas
Crop Science	
Analytical Service Laboratory	W. W. Weeks
Moving the "NC E-Z" Tobacco Breeding Method to Commercial Application	E. A. Wernsman
Remobilization of Nitrogen During Growth and Senescence of Flue-Cured Tobacco	R. C. Long

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Activity	Investigator(s)
Crop Science (continued)	
Leaf Surface Chemistry of *Nicotiana*: Rapid Methods for the Analysis of Component Classes	D. A. Danehower
Genetic Studies of Resistance to Tobacco Vein Mottling, Tobacco Etch Virus and Potato Virus Y on Burley Tobacco Using *in vitro* and Conventional Breeding Methodologies	R. C. Rufty
No-Till Tobacco	A. D. Worsham W. David Smith
Investigation of the Effects of Aerial CO_2 Enrichment on Growth and Development of Tobacco and on Tobacco Leaf Quality	T. W. Rufty, Jr.
Chemical and Smoke Panel Comparison of Popular Tobacco Varieties Grown from 1940-1984	W. W. Weeks J. F. Chaplin
Isozyme Variation Among and Within *Nicotiana* Species	V. A. Sisson
Genetic and Breeding Studies for Bacterial Wilt Resistance in Tobacco	G. R. Gwynn
Production of Nullisomics of *N. tabacum*	S. M. Reed
Economics and Business	
Explanation of the Size and Structure in Tobacco Farming: Including an Analysis of Potential Changes	D. A. Sumner
Entomology	
Population Dynamics of the Tobacco Flea Beetle and its Effect on Tobacco Yield	E. P. Lampert
Effects of Host-Plant Health on the Bionomics of the Green Peach Aphid	E. P. Lampert
Soil Arthropods and Nematodes Associated with No-Tillage Practices in Tobacco	G. J. House
Ovipositional Response of Tobacco Budworm Moths to *Nicotiana* Species	D. M. Jackson

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Activity	Investigator(s)
Entomology (Pesticide Residue Research Laboratory)	
Residue Studies with EBDC Fungicides on Flue-Cured Tobacco	T. J. Sheets C. E. Main
Residues of Prime+ in Flue-Cured Tobacco and Paarlan and Prime+ in Tobacco Soils	T. J. Sheets H. Seltmann
Effects of Dicamba, Picloram, Trichlopyr, and 2,4-D on Growth, Yield, Quality, and Value of Flue-Cured Tobacco	T. J. Sheets A. D. Worsham
Genetics	
Genetic Modification of Tobacco	D. F. Matzinger
Plant Pathology	
Development of Granville Wilt Nursery	M. E. Daub H. D. Shew
Biological Control and Genetic Studies of Bacterial Wilt in Naturally Infested Tobacco Fields	E. Echandi H. W. Spurr, Jr. G. R. Gwynn
Epidemiology of the Tobacco Black Shank Disease	H. D. Shew
Epidemiology of Tobacco Blue Mold	C. E. Main
Transfer of Resistance Genes from *Nicotiana reganda* to *Nicotiana tabacum* by Protoplast Fusion	M. E. Daub A. E. Jenns
Control of Flue-Cured Tobacco Diseases by Means of Cultural Practices, Varietal Resistance, and Chemical Application	N. T. Powell
Tobacco Blue Mold - Identification of Pathogenic Races	H. W. Spurr, Jr.
Alternative Fungicides and Strategies for Control of Blue Mold on Burley Tobacco	P. B. Shoemaker

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Activity	Investigator(s)
Plant Pathology (continued)	
Investigations on the Effect of Ozone on Burley Tobacco Infected with Tobacco Etch Virus or Tobacco Vein Mottling Virus	R. A. Reinert R. C. Rufty G. V. Gooding, Jr.
Cross Protection with Potato Virus Y	G. V. Gooding, Jr.
Cross Protection with Tobacco Mosaic Virus	G. V. Gooding, Jr.
Breeding for Resistance to Tobacco Vein Mottling Virus, Tobacco Etch Virus and Potato Virus Y	G. V. Gooding, Jr.
Soil Science	
Tobacco Root Distributions in No-Tilled and Conventionally Tilled Soils	M. J. Vepraskas G. D. Hoyt G. S. Miner
Tillage Systems for Burley Tobacco Production	G. D. Hoyt
Soil Management Practices for Improved Water Conservation, Erosion Control and Tobacco Production	D. K. Cassel G. R. Bathke H. P. Denton
Evaluation of Methods of Determining the Fertilizer Requirements of Varying Soil Types and Promotion of Use of Soil Test in Production of Quality Burley Tobacco	J. E. Shelton
Nitrogen Assimilation by Tobacco as Regulated by Carbohydrate Status	R. J. Volk T. W. Rufty, Jr.
Critical Carbohydrate and Nitrogen Levels in Tobacco Plants as Related to Floral Initiation	C. D. Raper, Jr. L. C. Tolley
Effect of Rate of Fertilizer Application on Salt Levels in the Soil and on Tobacco Transplant Production Under Plastic Plantbed Covers	G. S. Miner

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gator(s)

Reinert
Rufty
Gooding, Jr.

Gooding, Jr.

Gooding, Jr.

Gooding, Jr.

Vepraskas
Hoyt
Miner

Hoyt

Cassel
Bathke
Denton

Shelton

Volk
Rufty, Jr.

Raper, Jr.
Tolley

Miner

Activity	Investigator(s)
General	
Director's Reserve for Emergencies and Reserve Research Assistantships	W. H. Johnson

Please keep in mind that the information presented in this annual report is NOT FOR PUBLICATION but merely represents preliminary findings which may later appear in published form. We trust that you will find the information presented herein of interest and value.

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